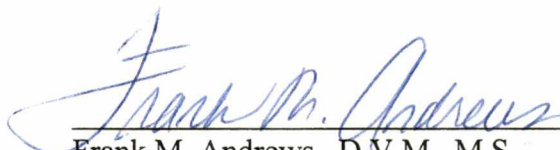


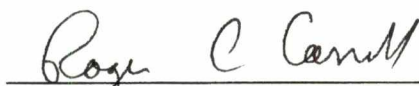
To the Graduate Council:

I am submitting herewith a dissertation written by Hilary Kay Matthews entitled "The Effect of Unilateral Nephrectomy on Glomerular Filtration Rate, Fractional Clearance of Urinary Electrolytes, Compensatory Renal Growth, and Plasma Growth Hormone Concentration in Late Gestation in the Fetal Lamb." I have examined the final copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Comparative and Experimental Medicine.

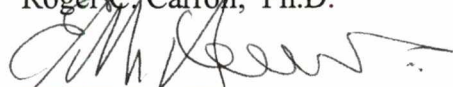


Frank M. Andrews, D.V.M., M.S.
Major Professor

We have read this dissertation
and recommend its acceptance:



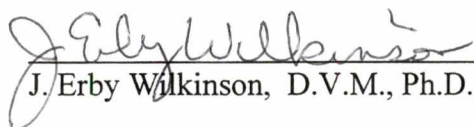
Roger C. Carroll, Ph.D.



Eddie S. Moore, M.D.

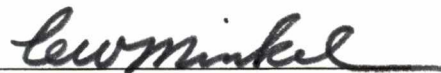


Susan E. Mulroney, Ph.D.



J. Erby Wilkinson, D.V.M., Ph.D.

Accepted for the Council:



Associate Vice Chancellor and
Dean of The Graduate School

**The Effect of Unilateral Nephrectomy on Glomerular
Filtration Rate, Fractional Clearance of Urinary Electrolytes,
Compensatory Renal Growth, and Plasma Growth Hormone
Concentration in Late Gestation in the Fetal Lamb**

A Dissertation Presented for the Degree of Doctor of Philosophy

The University of Tennessee, Knoxville

Hilary Kay Matthews

December 1996

DEDICATION

This dissertation is dedicated to my parents Edward J. and Helen L. Matthews, to whom I owe most of what I am today. You have provided me with educational opportunities and instilled in me the ability to know that with hard work, anything is possible. You are truly "the wind beneath my wings".

To my husband, Rolland, for his love, support and patience.

To Woody, my constant companion and provider of unconditional support.

Stately, kindly, lordly friend condescend here to sit by me, and turn glorious eyes that smile and burn..... Algernon Charles Swinburne

ACKNOWLEDGMENTS

I would like to express my gratitude and sincere thanks to the following individuals:

Dr. Eddie S. Moore, for providing me the wonderful opportunity to pursue this degree. If not for your support both professional and personal, I never could have completed this program. I will be forever grateful for all you did on my behalf.

Dr. Frank M. Andrews, my mentor and friend, for your invaluable help in seeing this process to fruition.

Dr. Susan E. Mulroney, for providing expert advice and technical assistance, valuable comments on the manuscript, and encouragement. I am forever indebted to you for your assistance.

Dr. Roger C. Carroll, for providing advice in statistical analysis and data management, and for providing moral support.

Dr. Erby Wilkinson, for providing materials and tissue analysis.

Sidney Holshouser and Margaret Buchholz, for providing excellent technical assistance. If not for the two of you this project would NEVER have been completed. Through difficult working situations we made the impossible possible. This dissertation is a reflection of a team effort and I will always be thankful for your dedicated hard work on this project .

Patricia Chadwick, for assistance in manuscript and slide preparation, and for helping me keep my sanity.

Dr. Dennis R. Geiser, for providing expert anesthesia assistance for my animals and for smoothing troubled waters. Gatorade!

Sharon Giles, Dean Knoll, and Scott Keaffaber, for providing excellent assistance with animal care.

Dr. Karla Matteson and Enid Bowlin, for providing materials and assistance for molecular biology techniques.

The Department of Pediatrics, for the opportunity to develop my research projects and successfully complete my studies.

The University of Tennessee Graduate School of Medicine, for partial funding of my research project.

To all my friends, especially Chris, Mary, Laurel, Joni, Brynn, Frank, and Pat for helping me keep my perspective and for providing much needed diversions.

Finally, to my four-legged friends, past, present and future, especially Mitzie, Scooter, Dexter, Chrissy, "B", Murphy, Jazz, Lucy, Taxi, Dunny, Neo, Rowdy, Shame, and Homer for helping me remember that there is a "normal" real life out there, and for reinforcing to me the reason I became a veterinarian.

ABSTRACT

It is well known that a reduction in renal mass results in compensatory renal growth (CRG) and functional adaptation of the remaining kidney. Age-related differences in response to unilateral nephrectomy (UNX) have been demonstrated. Immature animals respond primarily with hyperplastic CRG and have greater functional adaptation. Adult animals respond primarily with hypertrophic CRG and do not have as complete functional adaptation. The initiating mechanism(s) of the response to UNX remains elusive. There are a paucity of conflicting reports describing the response of the fetus to UNX, and there are no reports examining possible initiating mechanisms of the response. A study was undertaken to examine the response of the late gestational ($> \text{day } 130$) ovine fetus to UNX and to examine the role of growth hormone (GH) as a possible initiator of compensatory adaptation. Glomerular filtration rate (GFR) in mls/min or mls/min/kg body weight was significantly greater in sham operated (control) versus UNX animals 24 and 72 hours post-UNX. There was no difference in GFR in mls/min/gm kidney weight between the two groups at 24 and 72 hours post-UNX. Fractional clearance of urinary electrolytes was increased (not significantly) 24 hours post-UNX versus control animals. By 72 hours post-UNX, values for fractional clearance of electrolytes had decreased and were similar to control animals. Urine production 24 and 72 hours post-UNX was not different from control animals. No significant increase in remnant kidney weight was found 24 or 72 hours post-UNX versus control animals. By 72 hours post-UNX remnant kidney weight did trend toward an increase (8.5%) over normal growth during this time. Protein and DNA content of the remnant

kidney as a measure of hypertrophy and hyperplasia, respectively, increased over extant kidney values by 72 hours post-UNX. Mean plasma GH concentration, total GH secreted, and the pulsatile nature of GH secretion was not different from control animals 24 or 72 hours post-UNX. It appears that the late gestational ovine fetus can undergo functional adaptation and CRG following UNX, but the response is of a lesser magnitude compared with the postnatal animal, and this adaptation appears to be GH independent.

TABLE OF CONTENTS

PART 1: INTRODUCTION

General Background	2
Review of Renal Physiology	2
Age-related Differences in Compensatory Renal Growth	6
Rationale and Research Aims	9
Rationale	9
Research Aims/Hypothesis	10
Literature Cited	11

PART 2: THE EFFECT OF UNILATERAL NEPHRECTOMY ON GLOMERULAR FILTRATION RATE AND FRACTIONAL CLEARANCE OF URINARY ELECTROLYTES

Abstract	14
Review of the Literature	16
Changes in Renal Blood Flow Following Reduction in Renal Mass	16
Changes in Glomerular Filtration Rate Following Reduction	17
in Renal Mass	
Changes in Fractional Clearance of Electrolytes Following Reduction	18
in Renal Mass	
Materials and Methods	22
Animal Instrumentation	22
Physiologic Studies	25
Analytical Methods	26
Statistical Analysis	27
Results	28
Glomerular Filtration Rate	28
24 hours post-uninephrectomy	28
72 hours post-uninephrectomy	29
Fractional Clearance of Urinary Electrolytes	29
24 hours post-uninephrectomy	29
72 hours post-uninephrectomy	30

Urine Production	31
24 hours post-uninephrectomy	31
72 hours post uninephrectomy	31
Discussion	32
Glomerular Filtration Rate	32
Fractional Clearance of Urinary Electrolytes	34
Urine Production	35
Literature Cited	42

PART 3: THE EFFECT OF UNILATERAL NEPHRECTOMY ON COMPENSATORY RENAL GROWTH

Abstract	46
Review of the Literature	47
Overview of Compensatory Renal Growth	47
Age-related Differences in Compensatory Renal Growth	48
Fetal Compensatory Renal Growth	49
Materials and Methods	52
Animal Instrumentation	52
Sample Collection	52
Analytical Methods	52
Statistical Analysis	53
Results	54
Normal Kidney Growth Rate	54
24 hours	54
72 hours	54
Change in Kidney Weight	54
24 hours post-uninephrectomy	54
72 hours post-uninephrectomy	55
Change in Kidney DNA and Protein Content	56
24 hours post-uninephrectomy	56
72 hours post-uninephrctectomy	58
Discussion	60
Normal Growth Rate	60
Change in Kidney Weight	61
Change in Kidney DNA and Protein Content	63

Literature Cited	71
------------------------	----

PART 4: THE EFFECT OF UNILATERAL NEPHRECTOMY ON PLASMA GROWTH HORMONE CONCENTRATION

Abstract	74
Review of the Literature	75
The Work Hypothesis	75
Circulating Stimuli	76
Growth Hormone	77
Secretory Pattern	77
Role in Compensatory Renal Growth	78
Materials and Methods	81
Animal Instrumentation	81
Sample Collection	81
Radioimmunoassay of Growth Hormone	81
Statistical Analysis	83
Results	84
Plasma Growth Hormone Concentration	84
24 hours post-uninephrectomy	84
72 hours post-uninephrectomy	84
Discussion	85
Literature Cited	89

PART 5: SUMMARY

Summary of Parts 2-4	93
Limits of the Paradigm	97
VITA	99

LIST OF TABLES

TABLE

PART 2: THE EFFECT OF UNILATERAL NEPHRECTOMY ON GLOMERULAR FILTRATION RATE AND FRACTIONAL CLEARANCE OF URINARY ELECTROLYTES

Table 1.	Glomerular Filtration Rate (GFR) 24 Hours 37 Post-Uninephrectomy
Table 2.	24 Hour Right Kidney Glomerular Filtration Rate (GFR) 37
Table 3.	Glomerular Filtration Rate (GFR) 72 Hours 38 Post-Uninephrectomy
Table 4.	72 Hour Right Kidney Glomerular Filtration Rate (GFR) 38
Table 5.	Fractional Clearance of Urinary Electrolytes 24 Hours 39 Post-Uninephrectomy
Table 6.	Fractional Clearance of Urinary Electrolytes 72 Hours 39 Post-Uninephrectomy
Table 7.	Urine Flow Rate 24 Hours Post-Uninephrectomy 40
Table 8.	Urine Flow Rate 72 Hours Post-Uninephrectomy 40

PART 3: THE EFFECT OF UNILATERAL NEPHRECTOMY ON COMPENSATORY RENAL GROWTH

Table 1.	Change in Kidney Weight 24 Hours Post-Uninephrectomy 65
Table 2.	Kidney Weight to Body Weight (%) 24 Hours Post-Uninephrectomy .. 65
Table 3.	Change in Kidney Weight 72 Hours Post-Uninephrectomy 66
Table 4.	Kidney Weight to Body Weight (%) 72 Hours Post-Uninephrectomy .. 66
Table 5.	Change in Kidney DNA and Protein Concentration 24 Hours 67 Post-Uninephrectomy

Table 6.	Total Kidney DNA and Protein Concentration 24 Hours	67
	Post-Uninephrectomy	
Table 7.	Change in Kidney DNA and Protein Concentration 72 Hours	68
	Post-Uninephrectomy	
Table 8.	Total Kidney DNA and Protein Concentration 72 Hours	68
	Post-Uninephrectomy	

PART 4: THE EFFECT OF UNILATERAL NEPHRECTOMY ON PLASMA GROWTH HORMONE CONCENTRATION

Table 1.	Change in Plasma Growth Hormone Concentration 24 Hours	88
	Post-Uninephrectomy	
Table 2.	Change in Plasma Growth Hormone Concentration 72 Hours	88
	Post-Uninephrectomy	

PART 1
INTRODUCTION

GENERAL BACKGROUND

It has been known from the time of Hippocrates and Leonardo da Vinci that the fetus has a functioning urinary system as fetal urine production was observed (Needham, 1931). Until recently, the study of fetal renal physiology has largely been a theoretical science since it was assumed that the placenta was solely responsible for maintenance of fetal homeostasis. It is now known that the fetal kidney produces hormones and growth factors, and contributes to fluid and electrolyte homeostasis, and acid-base balance (Robillard et al., 1992). The importance of studying normal fetal renal function and the response the fetal kidney undergoes with perturbation is now considered essential knowledge for the care of premature infants and identification and management of fetal renal abnormalities.

Review of Renal Physiology

Normal renal function is essential for maintaining body homeostasis because the kidneys serve to filter the plasma and remove substances from the plasma at a variable rate depending on the need. The kidneys clear unwanted substances from the plasma filtrate into the urine for excretion, and reabsorb substances that are needed. To maintain homeostasis, excretion and intake must be matched, and it is the kidney that adjusts its excretion rates to match intake. Not only does the kidney regulate water and electrolyte balance, but it also has a role in excretion of metabolic waste products and foreign substances, regulation of arterial pressure and acid-base balance, and erythrocyte, 1,25-dihydroxy vitamin D₃, and glucose production (McDougal, 1996).

Blood flow to the kidneys in the postnatal animal is approximately 20-25% of the cardiac output. Blood enters the kidney via the renal artery and traverses the interlobar,

arcuate and interlobular arteries and afferent arterioles which then lead to the glomerular capillaries in the glomerulus. Fluids and solutes (except plasma proteins) are filtered at the glomeruli to begin the process of urine formation (McDougal, 1996).

Urine formation is the result of filtration from the glomerular capillaries into the nephron, which is the functional unit of the kidney. The plasma filtrate initially enters the glomerulus at Bowman's space and then flows through the nephron segments in the order of proximal tubule, descending and ascending loop of Henle, distal tubule, connecting tubule, cortical and medullary collecting tubule, and renal papillae to reach the ureter and then bladder. The final fluid composition of the urine is the result of glomerular filtration and reabsorption and secretion of substances from the tubules.

Plasma, water, and nonprotein crystalloids are separated from the blood cells and protein at the glomerulus by the process of ultrafiltration. The forces involved in ultrafiltration are the hydrostatic pressure within the glomerular capillaries, permeability of the glomerular membrane, oncotic pressure of the plasma and hydrostatic pressure in Bowman's space (McDougal, 1996).

The ability of the tubules to increase the rate of reabsorption in response to increased tubular load is known as glomerulotubular balance. The mechanism of glomerulotubular balance allows the rate of reabsorption of substances to increase as the filtered load increases even though the percent of glomerular filtrate reabsorbed in the proximal tubule remains constant. This mechanism is important because it prevents overloading of the more distal tubule segments as the filtered load increases (McDougal, 1996).

Another important feedback mechanism that is intrinsic to the kidneys that keeps renal blood flow and glomerular filtration rate (GFR) relatively constant despite marked changes in systemic arterial blood pressure is autoregulation. Autoregulation preserves renal blood flow so that GFR is held relatively constant to allow precise control of renal excretion of water and solutes (McDougal, 1996).

Glomerular filtration rate is the amount of fluid arriving into Bowman's space with time. Glomerular filtration rate is calculated by measuring the urine and plasma concentration of a substance that is freely filtered through the glomerulus and is neither reabsorbed or secreted by the tubules. Inulin, a polymer of fructose that is found in dahlia tubers, is the gold standard for measurement of GFR (McDougal, 1996).

Renal function in the late gestation fetus is present, although it is not of the same magnitude as in the postnatal animal. It is known that in the late gestation fetus, amniotic fluid is primarily regulated by fetal urine production. The fetal kidney is also essential for normal growth and development as anephric individuals are growth retarded and display abnormalities of bone formation (Nigam et al., 1996). The fetal kidney is also known to contribute to the maintenance of cardiovascular integrity, fluid and electrolyte and acid-base balance, and hormone synthesis (Smith et al., 1992).

Fetal renal blood flow is only 2-4% of the cardiac output, and is associated with a high renal vascular resistance and low filtration fraction compared with postnatal individuals (Smith et al., 1992). However, the mechanism of autoregulation of renal blood flow is present in the fetus (Smith et al., 1992).

Fetal GFR is also of a lesser magnitude than the postnatal animal, but GFR does increase with gestational age. The increase in fetal GFR correlates with fetal age and body weight. Maturation of GFR is due to completion of nephrogenesis, reduction in renal vascular resistance, increased function of the superficial nephrons, and moderation of the forces of ultrafiltration (Smith et al., 1992). Increases in GFR prior to birth are due primarily to the addition of new nephron units, with the remaining aforementioned causes responsible for increases in GFR after birth (Smith et al., 1992).

Fractional clearance of urinary electrolytes is greater in the fetus than in the postnatal animal. The reason for this difference is not known, but may be due to the large extracellular volume of the fetus or insensitivity of the fetus to aldosterone (Smith et al., 1992). It is known that glomerulotubular balance is present in the fetus, so imbalance of this mechanism is not thought to be responsible for the greater renal clearance of electrolytes in the fetus (Smith et al., 1992). Urinary phosphate concentration is found to be low in the fetus, and the mechanism for this is not known (Smith et al., 1992).

Even though the placenta performs the majority of the homeostatic functions in the fetus that are performed by the kidney in the postnatal period, the fetal kidney is functional, albeit at a lesser magnitude compared with the postnatal period. The fetal kidney can make adjustments in the renal excretion of substances, suggesting the major influences on fetal renal function are those directed towards maintaining its own internal composition (Lumbers, 1983).

The area of fetal renal development and physiology was greatly advanced with the advent of the chronic sheep preparation (Gresham et al., 1971). This system allows for

venous, arterial and urinary catheter placement, and perturbation of the fetal kidney(s) with return of the fetus to the normal uterine environment. Repeated measurements in the unanesthetized fetus over time are thus possible, allowing for the study of fetal renal function and the response of the fetus to experimental perturbation free from acute surgical and anesthetic stresses. However, the study of renal physiology in fetal animals is still technically difficult because the fetal animal is not in a steady state, and surgical procedures are less well tolerated than in the postnatal animal. The main drawback of using this system is not related primarily to the technical aspects so much as to accounting for the large variability between fetal animals and to the establishment of normal fetal growth rate.

The fetal lamb has been the preferred animal model to study human renal physiology because renal development of the lamb parallels that of man. Additionally, kidney and body weights of the two are similar, and the size of the fetal lamb permits repeated sampling of blood and/or urine over several hours and even days. Because it is possible to instrument and surgically manipulate fetal lambs in early gestation, long-term study of perturbations created early in the development process are possible.

Age-related Differences in Compensatory Renal Growth

It has also been known for centuries that removal, damage, or absence of one kidney results in adaptive changes in the remaining kidney (Nowinski, 1969). These adaptive or compensatory changes take the form of increased renal mass, known as compensatory renal growth (CRG), and function of the remaining kidney (Malt, 1969). Compensatory changes have been thought to be an appropriate response to reduced renal mass that allows the

individual to continue to meet the metabolic demands of the body, and thus body function may continue without impairment.

However, there appears to be an age-related difference in the consequences of compensatory changes in response to reduced renal mass. Studies in animals and man have demonstrated that the compensatory response following uninephrectomy (UNX) is greater in the young (MacKay et al., 1932; Kaufmann et al., 1975; Chevalier, 1992a; 1992b). Although the young appear to have an adaptive advantage, their long-term outcome may actually be worse. Unilateral renal agenesis (URA) is a clinical condition that demonstrates how greater adaptation to reduced renal mass in the young may actually be maladaptive. Individuals with URA are born with a solitary kidney, therefore; having a long interval in which their remaining hyperfunctioning kidney is exposed to adaptive changes. It was thought that URA was a benign condition. However, long-term studies of these individuals now demonstrate that children with URA develop a significant risk for focal segmental glomerulosclerosis, hypertension, proteinuria, and renal failure as adults (Kiprov et al., 1982; Argueso et al., 1992). Findings in rats have shown that male gender and young age at the time of UNX are risk factors for early renal damage, especially focal segmental glomerulosclerosis, with subsequent functional loss (O'Donnell et al., 1985; Okuda et al., 1987; Provoost et al., 1991; Nagata et al., 1992).

A recent study performed at The University of Tennessee Medical Center found the incidence of some type of obstructive uropathy to be one in 211 live births (Malagon and Moore, 1994). Therefore, congenital conditions that may lead to a compensatory renal response are not infrequent findings. The impact on the health of the child and the emotional

and financial burden of these problems on the family are potentially devastating. Therefore, a better understanding of the initiating mechanism(s) and consequences of compensatory adaptation in the fetus will help clarify clinical decisions and patient counseling in such cases.

RATIONALE AND RESEARCH AIMS

Rationale

Much basic information is known regarding compensatory adaptation to reduced renal mass in the adult and young individual. Because there are age-related differences between these two groups in the type and magnitude of CRG and the mechanisms stimulating CRG, fetal CRG may be similar or different from the response seen in the young and adult animal. There are only a few reports in the literature regarding compensatory renal adaptation in the fetus, and none of these studies addresses the total response to a reduction in renal mass including functional adaptation, CRG, and potential initiators of the compensatory response (Malt and Lemaitre, 1969; Goss and Walker, 1971; Moore et al., 1979; Peters et al., 1993). Because of the greater possibility of potentially devastating effects on individuals that suffer nephron loss in the early stages of development, the chronic sheep model was chosen as a way to investigate the manner and extent of CRG and functional adaptation in the fetus. Identification of potential initiator(s) of CRG, such as growth hormone (GH), were also investigated since this may provide an avenue for therapeutic intervention. By examining the response the fetus undergoes to a reduction in renal mass in-utero, questions regarding the magnitude of CRG and functional adaption, and potential triggers of such a response can begin to be answered.

Research Aims/Hypothesis

Using UNX in the chronic sheep model the following specific aims were examined:

1. Documentation of normal renal function parameters including glomerular filtration rate, fractional clearance of urinary electrolytes, and urine flow rate.

Hypothesis: By using standard clearance techniques, renal function can be fully documented in the ovine fetus.

2. Documentation of changes that occur in renal function in response to UNX in the ovine fetus.

Hypothesis: Because the ovine fetal kidney does have functional capacity albeit it at a reduced level compared with the postnatal animal, it is expected that renal function will undergo compensatory adaptation in response to UNX.

3. Documentation of the degree of renal hypertrophy and/or hyperplasia that occurs in the ovine fetus in response to UNX.

Hypothesis: It is expected that the remnant fetal kidney will undergo CRG in response to UNX as demonstrated by hypertrophy and/or hyperplasia.

4. Quantitation of plasma GH concentration by radioimmunoassay in the sham operated ovine fetus and in the ovine fetus undergoing UNX.

Hypothesis: Because GH secretion following UNX does not change in the immature rat, it is expected that no change in GH secretion will occur in the ovine fetus following UNX.

LITERATURE CITED

- Argueso LR, Ritchey ML, Boyle ET et al. Prognosis of patients with unilateral renal agenesis. *Pediatr Nephrol.* 6:412-416, 1992.
- Chevalier RL. Response of the developing kidney to nephron loss. In: *Pediatric Kidney Disease.* Edelmann EM ed. Little & Brown, Boston. 443-458, 1992.
- Chevalier RL. The response to nephron loss in early development. In: *Fetal and Neonatal Physiology.* Polin RA and Fox WW eds. WB Saunders Company, Philadelphia. 2:1264-, 1992.
- Goss RJ, Walker MJ. Compensatory renal hypertrophy in fetal rats. *J Urol.* 106:360-362, 1971.
- Gresham EL, Rankin JH, Makowski EL et al. An evaluation of fetal renal function in a chronic sheep preparation. *J Clin Invest.* 51:149-156, 1972.
- Kaufman JM, Hardy R, Hayslett JP. Age dependent characteristics of compensatory renal growth. *Kidney Int.* 8:21-26, 1975.
- Kiprov DD, Colvin RB, McCluskey RT. Focal and segmental glomerulosclerosis and proteinuria associated with unilateral renal agenesis. *Lab Invest.* 46:275-282, 1982.
- Lumbers ER. A brief review of fetal renal function. *J Dev Physiol.* 6:1-10, 1983.
- McDougal WS. The kidney. In: *Adult and Pediatric Urology.* Gillenwater JY, Grayhack JT, Howards SS, Duckett JW eds. Mosby, St. Louis. 1:617-642, 1996.
- Malagon M, Moore ES. Hydronephrosis in the newborn: is it benign? *J Am Soc Nephrol.* 5:649, 1994.
- Malt RA, Lemaitre DA. Nucleic acids in fetal kidney after maternal nephrectomy. *Proc Soc Exp Biol Med.* 130:539-542, 1969.
- Malt RA. Compensatory growth of the kidney. *N Engl J Med.* 280:1446-1459, 1969.
- Moore ES, deLeon LB, Weiss LS et al. Compensatory renal hypertrophy in fetal lambs. *Pediatr Res.* 13:1125-1128, 1979.
- Nagata M, Scharer K, Kriz W. Glomerular damage after uninephrectomy in young rats. I. Hypertrophy and distortion of capillary architecture. *Kidney Int.* 42:136-147, 1992.

Needham J. Chemical embryology. Cambridge Univ Press, Cambridge UK, Vol 1, 1931.

Nigam SK, Aperia AC, Brenner BM. Development and maturation of the kidney. In: The Kidney. Brenner BM ed. WB Saunders Company, Philadelphia. 1:72-98, 1996.

Nowinski WW. Early history of renal hypertrophy. In: Compensatory Renal Hypertrophy. Nowinski WW and Goss RJ eds. Academic Press, New York. 1-8, 1969.

O'Donnell MP, Kasiske BL, Raij L et al. Age is a determinant of the glomerular morphologic and functional processes to chronic nephron loss. J Lab Clin Med. 106:308-313, 1985.

Okuda S, Motomura K, Sanai T et al. Influence of age on determination of the remnant kidney in uninephrectomized rats. Clin Sci. 72:571-576, 1987.

Robillard LE, Smith FG, Smith FG. Developmental aspects of renal function during fetal life. In: Pediatric Kidney Disease. Edelmann CM ed. Little, Brown & Company, Boston. 3-18, 1992.

Smith FG, Nakamura KT, Segar JL et al. Renal function in utero. In: Fetal and Neonatal Physiology. Polin RA, Fox WW eds. WB Saunders Company. 2:1197-1195, 1992.

PART 2

THE EFFECT OF UNILATERAL NEPHRECTOMY ON GLOMERULAR FILTRATION RATE AND FRACTIONAL CLEARANCE OF URINARY ELECTROLYTES

ABSTRACT

Following a reduction in renal mass, it is known that adaptive changes occur in glomerular filtration rate and tubular function in order to maintain homeostasis. These adaptive changes following a reduction in renal mass have been well documented in the adult and immature animal using the model of unilateral nephrectomy (UNX). It is still controversial if UNX in the fetus produces a functional compensatory response as the placenta is largely responsible for waste excretion. Therefore, the ability of the late gestational fetal ovine kidney to undergo an acute functional adaptation to UNX was examined. Glomerular filtration rate (GFR) in control animals (3.84 ± 0.42 mls/min) was significantly greater than UNX animals (2.23 ± 0.28 mls/min) 24 hours post-UNX at the $P < 0.05$ level. The same held true for comparison of GFR in mls/min/kg body weight 24 hours post-UNX. However, when GFR was expressed in mls/min/gm kidney weight, there was no difference between the control (0.17 ± 0.02 mls/min/gm kidney weight) and UNX (0.20 ± 0.02 mls/min/gm kidney weight) groups. Glomerular filtration rate in control animals (3.45 ± 0.32 mls/min) was significantly greater than UNX animals (1.98 ± 0.49 mls/min) 72 hours post-UNX at the $P \leq 0.05$ level. The same held true for comparison of GFR in mls/min/kg body weight 72 hours post-UNX. When GFR was expressed in mls/min/gm kidney weight, there was no difference between the control (0.16 ± 0.01 mls/min/gm kidney weight) and UNX (0.17 ± 0.03 mls/min/gm kidney weight). Additionally, in UNX animals, GFR expressed as mls/min/gm kidney weight was significantly greater than control animal single kidney GFR expressed as mls/min/gm right kidney at 24 and 72 hours post-UNX ($P < 0.05$). Fractional clearance of urinary sodium, potassium, chloride, and phosphate all

increased 24 hours post-UNX over control values, but the increase was not significant. By 72 hours post-UNX, fractional clearance of electrolytes had greatly decreased versus 24 hour excretions, and values were much more similar to control values. At 72 hours post-UNX, there was no significant difference between control and UNX animal clearance values. Urine flow rates 24 hours post-UNX in control (11.77 ± 1.88 mls/20 min) and UNX (9.06 ± 1.45 mls/20 min) groups were not significantly different. At 72 hours post-UNX, urine flow rates between control (11.34 ± 2.65 mls/20 min) and UNX (7.03 ± 1.79 mls/20 min) were not significantly different. This study demonstrates that the fetal kidney does functionally adapt to a reduction in renal mass by increasing GFR per amount of remaining functioning renal mass. Additionally, glomerulotubular imbalance is initially disturbed following a reduction in renal mass as evidenced by the increased fractional excretion of electrolytes 24 hours post-UNX, but by 72 hours, balance is restored.

REVIEW OF THE LITERATURE

Following a reduction in renal mass, it is necessary for adaptive changes to occur in glomerular and tubular function in order to maintain homeostasis. The compensatory changes in renal function that occur in response to a reduction in renal mass have not been fully characterized. The adaptive mechanisms have been primarily documented in the adult animal, with investigation of the response to a lesser extent in the young or immature animal. Little is known about the functional changes, if any, that occur in the fetus in response to a reduction in renal mass.

Changes in Renal Blood Flow Following Reduction in Renal Mass

The changes that occur in renal blood flow (RBF) following an acute reduction in renal mass, such as unilateral nephrectomy (UNX) are varied. Early studies in the literature showed either no change or a reduction in RBF in response to UNX (Peters, 1963; Allison et al., 1973; Potter et al., 1974; Humphreys et al., 1978). These studies used clearance techniques to measure RBF, therefore; small changes in RBF were not readily detected. With the use of electromagnetic flow probes, other investigators have demonstrated a 13-30% increase in RBF that can occur as early as 30 minutes following UNX (Krohn et al., 1970; Chevalier and Kaiser, 1985).

In contrast to acute changes following UNX, most investigators agree that over time following UNX, RBF in the remaining renal tissue increases. The increase in RBF occurs predominately in the inner cortex and results from vasodilation (Kaufman et al., 1975). Comparison of the magnitude of the increase in RBF in response to 75% nephrectomy in one

day-old puppies versus eight week-old puppies demonstrated that the adaptive response was greater and more complete in the more immature animal (Aschinberg et al., 1978). Studies in guinea pigs have also demonstrated a compensatory increase in RBF in response to UNX at birth that is up to 70% above that due to normal growth and development (Chevalier, 1983). In the immature animal, it appears that the increase in RBF in response to reduction in renal mass occurs through a mechanism that is an acceleration of the normal developmental pattern of increased RBF.

Changes in Glomerular Filtration Rate Following Reduction in Renal Mass

Glomerular filtration rate (GFR) is agreed by most investigators to increase in response to an acute reduction in renal mass (Potter et al., 1974; Diezi et al., 1976; Provoost and Molenaar, 1980). However, some investigators have found no increase in GFR in response to an acute reduction in renal mass (Peters, 1963; Allison et al., 1973).

In contrast to the somewhat conflicting findings of the change in GFR in response to an acute reduction in renal mass, the chronic response to reduction in renal mass is an increase in GFR over time that is proportional to the remaining kidney weight (Galla et al., 1974; Aschinberg et al., 1978). Additionally, it is known that increases in GFR after a reduction in renal mass are greater in the young than in the adult (Aschinberg et al., 1978).

In the immature animal, deep nephrons are the primary functional nephrons with subsequent involvement of more superficial nephrons over time. Therefore, a centrifugal pattern of nephron maturation occurs with normal postnatal renal maturation (Spitzer and Edelmann, 1971; Spitzer and Brandis, 1974). Animals undergoing nephrectomy at or shortly after birth experience an accelerated centrifugal pattern of maturation with rapid recruitment

of more superficial nephrons (Chevalier, 1982). That is, the compensatory increase in superficial nephron GFR is greater than that of deeper nephrons. This is in contrast with adult animals in which a reduction in renal mass results in an equal compensatory increase in GFR in both superficial and juxtamedullary nephrons (Carriere and Brunette, 1977; Buerkert et al., 1979). It is still unknown if the increase in GFR precedes, follows, or occurs simultaneously with compensatory renal growth of the remaining renal tissue that also occurs with a reduction in renal mass.

There are few studies in the literature documenting changes in GFR in response to UNX in the fetus. In fact, to the author's knowledge only one study exists (Moore et al., 1979). Because the superficial nephrons of the fetus are essentially nonfunctional, it may be expected that any increase in GFR in response to UNX in the fetus would be minimal (Chevalier and Kaiser, 1985; Chevalier 1992). Additionally, because the placenta is primarily responsible for homeostasis during the fetal period, it may be unnecessary for any increase in GFR to occur. In the study by Moore et al., renal function in the 85-100 day fetal lamb undergoing UNX was studied. Seventy-two hours after UNX, these investigators found no significant increase in GFR. However, the GFR was increased 6-7% over sham operated animals, demonstrating a trend toward functional adaptation (Moore et al., 1979). This study demonstrates that the fetus is capable of responding with increased renal function , albeit at a lesser magnitude than the postnatal animal, to a reduction in renal mass in-utero.

Changes in Fractional Clearance of Electrolytes Following Reduction in Renal Mass

Urine flow and urinary sodium excretion have been observed to abruptly increase in response to reduced renal mass (Peters, 1963; Allison et al., 1973; Potter et al., 1974; Diezi

et al., 1976). This response has been termed compensatory adaptation (Peters, 1963). An increase in potassium excretion has also been observed following UNX (Ribstein and Humphreys, 1984). The increase in excretion rates is thought to be a consequence of reduced fractional reabsorption in the proximal tubule with absolute proximal reabsorption remaining constant despite the increased filtration rate (Dirks and Wong, 1978; Diezi et al., 1978; Shirley and Walter, 1991). However with increasing time post-UNX, fractional reabsorption becomes similar to control animals (Hayslett et al., 1968). A more recent study by Shirley and Walter documented changes in sodium and potassium excretion in the acute and chronic stages following UNX (Shirley and Walter, 1991). These investigators found that at all times post UNX, there was increased fluid delivery to the distal tubule, marked potassium secretion from the distal tubule, and no increase in sodium delivery to the late distal tubule, suggesting that natruresis was a result of reduced sodium reabsorption in the collecting ducts and/or increased sodium delivery from deep nephrons. This study also found that acutely post UNX, excretion rates of sodium and potassium doubled over controls, but by 30 days post UNX, proximal reabsorption had increased 40% and glomerulotubular balance was restored. Thus, because hyperfiltration of remaining nephrons following a reduction in renal mass occurs over time, fractional clearance of sodium per nephron must increase in the acute period if sodium balance is to be maintained (Hayslett, 1979).

It is known that in the fetal sheep kidney, fractional clearance of sodium and chloride is greater than in the adult, but the rate of excretion decreases as the fetus nears term (Robillard et al., 1977). This may be due to the large extracellular volume in the fetus (Robillard et al., 1992). In the fetal sheep, it is known that glomerulotubular balance does

exist, so an imbalance in this mechanism cannot explain the increased urinary sodium excretion (Lumbers et al., 1988). Other postulated mechanisms for increased sodium excretion in the fetus include high circulating levels of cortisol, prostaglandin E₂ and I₂, and atrial natriuretic peptide, low oxygen delivery to the fetal kidney, and a relative tubular insensitivity to aldosterone (Robillard et al., 1992).

Potassium excretion in the fetal sheep increases as the fetus approaches term (Robillard et al., 1980). The mechanism for this is not known, but may be related to increased flow through the distal nephron, increased ATPase activity which is necessary for potassium secretion, and increased sensitivity of the nephron to aldosterone (Robillard et al., 1992).

Phosphate reabsorption by the fetal sheep kidney is between 60-100% (Hill and Lumbers, 1988). The mechanism for this high rate of phosphate reabsorption remains unknown. However, in the immature rat, growth hormone (GH) stimulates phosphate reabsorption, and since GH levels are higher in the fetus than in post-natal life, the high rate of phosphate reabsorption in the fetus may be GH related (Mulroney et al., 1989).

Only one report in the literature addresses the changes that occur in excretion of substances in the fetus. Moore et al. found that excretion of phosphate increased significantly over control values 72 hours post UNX in the 85-100 day fetal sheep (Moore et al., 1979). Urinary excretion of other substances was not examined in this study. However, this study did address the change in urine production that occurs following UNX and found an increase in the urine flow rate of 92.3% over control values 72 hours post-UNX.

In summary, the functional changes that occur following reduction in renal mass have been partially characterized in the young and adult animal. However, these changes have not been well documented in the fetus, despite the implications a reduction in renal mass during development may have on the long-term survival of the individual. Thus, a study was undertaken to document the changes that occur in renal function in the late term ovine fetus 24 and 72 hours post-UNX.

MATERIALS AND METHODS

Animal Instrumentation

Pregnant Dorset and mixed-breed Dorset ewes were obtained from a local source and the gestational age was based on planned breeding dates. The fetuses used in the study were greater than 130 days gestation, term 150 days. Upon arrival, ewes were given a thorough physical examination and dewormed with albendazole per os. Additional health screening tests consisted of complete blood count, plasma fibrinogen, serum biochemical panel, urinalysis, leptospirosis titers, and Q-fever and brucella serology. Only ewes that passed the physical examination and all health screening tests were admitted to the study. All ewes were fed a commercial sheep feed and allowed free access to grass and alfalfa hay, mineral block, and water. A seven day acclimation period was allowed prior to surgical instrumentation.

Prior to surgery, the ewes were fasted for 24 hours. A jugular catheter was placed prior to induction of anesthesia. Induction of anesthesia was achieved with diazepam (0.15 mg/kg IV) followed by ketamine (4 mg/kg IV). All ewes were then intubated and placed on isoflurane to maintain anesthesia. Mechanical ventilation was used to ensure physiologic fetal blood gases. Direct arterial pressure, end-tidal CO₂, percent O₂ saturation, and arterial blood gases were performed to monitor the ewe during the surgical procedure. A heating pad under the ewe and a heat lamp placed directly over the surgical site were used to keep the fetal lamb warm. After a surgical plane of anesthesia was attained and the ventral abdomen sterilely prepared, a 20 cm incision through the skin, subcutaneous tissue and linea alba was made starting at the umbilicus and extending caudally. The gravid uterus was then identified

and an incision in an avascular part of the uterus was made over the head of the tail of the fetus. Both pelvic limbs of the fetus were then exteriorized from the uterus and wrapped in warm saline soaked towels. The fetus was periodically lavaged with warm 0.9% NaCl throughout the surgical procedure to maintain warmth. The proximal two-thirds of the fetus remained submerged in amniotic fluid at all times during the procedure, and there was no onset of spontaneous respiration by the fetus.

The fetal femoral artery and vein were catheterized via direct cut-down. Tygon catheters (Tygon S-54-HL 1.02 x 1.78 mm, Baxter Scientific, Stone Mountain, GA) were inserted approximately 10 cm and secured with 2-0 silk using a Chinese finger-trap pattern. The muscle, subcutaneous tissue and skin over the catheter site were closed in a routine fashion. The bladder was catheterized with Tygon tubing (Tygon S-50-HL 2.38 x 3.97 mm, Baxter Scientific, Stone Mountain, GA) via a suprapubic approach. The catheter was inserted 5 cm and secured with a purse-string pattern. Linea alba, subcutaneous tissues and skin over the site were then closed in a routine fashion. Nephrectomized fetal lambs had the left kidney removed via a flank approach. The renal artery, vein, and ureter were double ligated with 0 silk. The flank incision was closed in a routine fashion. Sham operated animals had their left kidney exteriorized and gently manipulated for one minute, the time approximating kidney removal in the nephrectomy group. The kidney was then replaced and the flank incision closed in a routine fashion.

Once all surgical instrumentation was complete, the fetus was returned to the uterus. Warm 0.9% NaCl was added to the uterus to replace amniotic fluid that was lost. Ceftiofur sodium was administered to the ewe (2.2 mg/kg IV), and 50 mg was put into the uterus

before closure. The uterus was closed with 1 chromic gut in a double inverting pattern with the fetal catheters exiting through the uterine incision. Approximately 5-7 cm of the catheters were tacked to the outside of the uterus. The remaining length of the catheters was tied to a 0.5 cm diameter metal trocar 60 cm in length that was used to exit the catheters out the right flank of the ewe. The exited catheters were secured with a purse-string suture, and the catheter ends with attached adaptors placed in a plastic sealable pouch. A heparin lock was placed in the venous and arterial catheters. The bladder catheter was tied onto itself so that no urine could drain from the catheter. The linea alba was closed in a simple continuous pattern with 2 proline. The skin was apposed with surgical skin staples.

At the end of the surgical procedure, the ewe was turned off gas anesthesia and weaned off the mechanical ventilator. Flunixin meglumine (2.2 mg/kg IV) was administered for additional analgesia at the time the gas anesthetic was discontinued. Once the ewe was breathing spontaneously, she was placed on a padded cart and her abdomen and jugular catheter site bandaged. The ewe was then rolled back to a stall and monitored until standing. Most ewes were standing and eating hay within 30 minutes from entry into their stall.

Post-operatively, all ewes were returned to their usual diet. Monitoring consisted of daily fetal blood gases, continuation of ceftiofur sodium IV twice daily, and physical examination of the ewe three times daily. Catheters in the ewe and fetus were handled in a sterile manner. Control animals were either single or twin pregnancies. Nephrectomized animals were from single pregnancies in order to eliminate any confounding influence from the twin.

Physiologic Studies

Physiologic Studies

Either 24 or 72 hours after surgery, the ewe was transferred to the study room in a small cart restricting her to the standing position. She was allowed free access to grain and hay during the study period. Lactated ringers solution was administered IV to the ewe at a maintenance rate (2 ml/kg/hr) throughout the study period. A fetal arterial blood gas was performed prior to the study period to ensure that the fetus was physiologic. Any fetus not in the normal range for arterial blood gas values was excluded from the study. A baseline sample of heparinized blood and urine was obtained prior to the start of each study.

Fetal glomerular filtration rate was determined using carboxyl- ^{14}C inulin (DuPont/NEN, Boston, MA) clearance. Prior to use, 1 m Ci of ^{14}C inulin was dissolved in 4 mls of 0.9% NaCl. Fifty microliter aliquots were frozen at -20 C for future use. At the time of use, a 50 μl aliquot was dissolved in 22 mls of 5% dextrose, specific activity 0.578 $\mu\text{Ci/ml}$, and then administered to the fetus as a 3 ml bolus IV followed by constant infusion at a rate of 3.4 mls per hour for three hours. Constant infusion was achieved by use of a Sage infusion pump (Sage Instruments, Boston, MA). After a one hour equilibration period, urine samples were collected from the fetal urinary catheter via gravity flow over six 20-minute periods. Collected urine volume from the six sample periods was recorded. Immediately after the 20-minute collection period, the urine was aliquoted into portions and frozen at -80 C for future electrolyte and protein analysis and for ^{14}C inulin scintillation counting. Approximately 2 mls of heparinized blood was collected from the arterial catheter midway through each urine collection period. The volume of withdrawn blood was replaced with warm 0.9% NaCl given IV at three times the volume of withdrawn blood. The blood samples were placed on ice

and centrifuged in a refrigerated centrifuge to separate the plasma. Plasma samples were frozen at -80 C for future electrolyte and protein analysis and ^{14}C inulin scintillation counting.

At the end of the collection periods the ewe was again anesthetized as before so that a section of fetal liver and the fetal kidney(s) and the left maternal kidney could be harvested for tissue analysis (see Part 3 Materials and Methods). Both the ewe and fetus were euthanized while under general anesthesia with a barbiturate and phenytoin overdose (Beuthanasia®-D Special 1ml/2.2kg IV; Schering-Plough Animal Health, Kenilworth, NJ).

Analytical Methods

Beta emission from the ^{14}C inulin in the plasma and urine samples was measured in triplicate in a liquid scintillation counter (Beckman Model LS-5000TD, Fullerton, CA). Plasma and urinary sodium and potassium were measured using a flame photometer (Instrument Laboratories Model IL-943, Lexington, MA). Plasma and urinary chloride were measured using a digital chlorometer (Haake Buchler Model DP-957M, Saddle Brook, NJ). Plasma and urinary phosphate was measured by the molybdate spectrophotometric method of Chen et al. (Chen et al., 1956). Plasma and urinary protein was measured by the spectrophotometric method of Bradford (Bradford, 1976).

Glomerular filtration rate was calculated using the following formula:

$$\text{GFR (mls/min)} = \frac{\text{Urine volume} \times (\text{urine } ^{14}\text{C inulin cpm} - \text{baseline cpm})}{\text{Time (min)} \times (\text{plasma } ^{14}\text{C inulin cpm} - \text{baseline cpm})}$$

Additionally, GFR was calculated in mls/min/kg and as mls/min/gm kidney weight.

Fractional clearance (Fe) of urinary sodium, potassium, chloride, and phosphate was

calculated as follows:
$$Fe_{[X]} = \frac{GFR \text{ (mls/min)} \times \text{Urine [X]} \times 20 \text{ min}}{\text{Plasma [X]} \times \text{Urine volume}}$$

Statistical Analysis

Significant differences between the means of the control (sham operated) and nephrectomized fetal lambs were determined by Student's *t* test. Significant differences between the means within groups was determined by paired Student's *t* test. Significance was determined at the $P \leq 0.05$ level. SigmaStat™ statistical software was used for all statistical analysis (Jandel Scientific, San Rafael, CA).

RESULTS

Glomerular Filtration Rate

24 hours post-uninephrectomy

Table 1¹ shows the changes in GFR 24 hours post-UNX. Values are expressed as mean $\pm$ standard error of the mean (SEM). Body weight in the control (sham operated) group was 3.93 ± 0.71 kg and weight in the nephrectomy group was 3.82 ± 0.29 kg. The difference in weights between the two groups was not statistically different. Glomerular filtration rate expressed as mls/min was 3.84 ± 0.71 in the control group and 2.23 ± 0.27 in the nephrectomy group. This represented a 72.2 % decrease in GFR in the nephrectomy group and was a statistically significant decrease ($P \leq 0.05$). Glomerular filtration rate expressed as mls/min/kg was 0.92 ± 0.09 in the control group and 0.57 ± 0.04 in the nephrectomy group. The difference between the two groups was 61.4 % and represented a statistically significant difference ($P \leq 0.05$). Expression of GFR in mls/min/gm total kidney weight was 0.17 ± 0.02 in the control group and 0.20 ± 0.02 in the nephrectomy group. The difference between the two groups was not significant at the $P \leq 0.05$ level.

Table 2 shows GFR expressed as mls/min/gm of right kidney. The calculated value in the nephrectomy group was 0.20 ± 0.02 which was 150% more than the calculated value of 0.08 ± 0.01 in the control group. This represented a statistically significant difference ($P \leq 0.05$).

¹ All Tables and Figures appear after the Discussion section.

72 hours post-uninephrectomy

Table 3 shows the changes in GFR 72 hours post-UNX. Values are expressed as mean $\pm$ SEM. Body weight in the control group was 3.33 ± 0.32 kg and weight in the nephrectomy group was 3.35 ± 0.43 kg. The difference in weights between the two groups was not statistically different. Glomerular filtration rate expressed as mls/min was 3.45 ± 0.47 in the control group and 1.98 ± 0.49 in the nephrectomy group. This represented a 74.2% decrease in the nephrectomy group was a statistically significant decrease ($P \leq 0.05$). Glomerular filtration rate expressed as mls/min/kg was 1.03 ± 0.07 in the control group and 0.59 ± 0.07 in the nephrectomy group. The difference between the two groups was 74.5% and represented a statistically significant difference ($P \leq 0.05$). Expression of GFR in mls/min/gm total kidney weight was 0.16 ± 0.01 in the control group and 0.17 ± 0.03 in the nephrectomy group. The difference between the two groups was not significant at the $P \leq 0.05$ level.

Table 4 shows GFR expressed as mls/min/gm of right kidney. The calculated value in the nephrectomy group was 0.17 ± 0.03 which was 112.5% more than the calculated value of 0.08 ± 0.01 in the control group. This represented a statistically significant difference ($P \leq 0.05$). A summary comparison of right kidney GFR 24 and 72 hours post-UNX is shown in Figure 1.

Fractional Clearance of Urinary Electrolytes

24 hours post-uninephrectomy

Table 5 shows values for fractional clearances of urinary electrolytes and concentration of urinary protein 24 hours post-UNX. Values are expressed as mean $\pm$ SEM.

Clearance of sodium increased from $8.38 \pm 1.95\%$ in the control group to $12.01 \pm 7.17\%$ in the nephrectomy group representing a 43.3% increase. Clearance of potassium increased from $33 \pm 6.52\%$ in the control group to $80 \pm 20.92\%$ in the nephrectomy group representing a 142.4% increase. Clearance of chloride increased from $8.89 \pm 2.2\%$ in the control group to $16.38 \pm 2.97\%$ in the nephrectomy group representing a 84.3% increase. Clearance of phosphate increased from $27.3 \pm 7.48\%$ in the control group to $28.4 \pm 11.46\%$ in the nephrectomy group representing a 4% increase. Urinary protein concentration increased from 0.38 ± 0.13 mg/ml in the control group to 0.48 ± 0.14 mg/ml in the nephrectomy group representing a 26.3% increase. None of these increases were statistically significant at the $P \leq 0.05$ level.

72 hours post-uninephrectomy

Table 6 shows values for fractional clearances of urinary electrolytes and concentration of urinary protein 72 hours post-UNX. Values are expressed as mean $\pm$ SEM. Clearance of sodium decreased from $7.16 \pm 2.49\%$ in the control group to $5.39 \pm 1.21\%$ in the nephrectomy group representing a 32.8% decrease. Clearance of potassium decreased from $41.1 \pm 12.42\%$ to $21.2 \pm 9.88\%$ in the nephrectomy group representing a 93.8% decrease. Clearance of chloride decreased from $7.48 \pm 3.01\%$ in the control group to 5.33 ± 2.03 in the nephrectomy group representing a 40.3% decrease. Clearance of phosphate increased from $20.8 \pm 11.9\%$ in the control group to $25.6 \pm 17.9\%$ in the nephrectomy group representing a 23.1% increase. Urinary protein concentration increased from 0.29 ± 0.08 mg/ml in the control group to 0.44 ± 0.21 mg/ml in the nephrectomy group representing a

51.7% increase. None of the changes in the nephrectomy group was statistically different from the control group at the $P \leq 0.05$ level.

Urine Production

24 hours post-uninephrectomy

Table 7 shows mean $\pm$ SEM values for urine production per 20 minute collection period 24 hours post-UNX. Urine production was 11.77 ± 1.88 mls/20 min in the control group and 9.06 ± 1.45 mls/20 min in the nephrectomy group. There was no statistical difference between the groups at the $P \leq 0.05$ level.

72 hours post-uninephrectomy

Table 8 shows mean $\pm$ SEM values for urine production per 20 minute collection period 72 hours post UNX. Urine production was 11.34 ± 2.65 mls/20 min in the control group and 7.03 ± 1.79 mls/20 min in the nephrectomy group. There was no statistical difference between the groups at the $P \leq 0.05$ level.

DISCUSSION

Glomerular Filtration Rate

Glomerular filtration rate is defined as the sum of the filtration rates of each functioning nephron and represents an index of functional mass. The results of the GFR data indicate that following UNX in the late gestational fetal lamb, GFR when expressed as mls/min or mls/min/kg does not increase. However, when GFR was expressed as ml/min/gm kidney weight, there was no significant difference between the control and nephrectomy groups, indicating the remaining nephrons in the UNX group had increased their function so that GFR was similar to the control animal. In fact, when GFR was expressed as mls/min/gm of right kidney, the UNX group had a statistically significant increase in GFR which was 150% and 112.5% greater 24 and 72 hours post-UNX, respectively. When comparing GFR in this manner, it is apparent that the remaining functional renal tissue in the UNX group is functioning at a much higher level than the comparison right kidney in the control group.

The control values for GFR in mls/min/gm of right kidney were obtained by halving the value in mls/min/gm kidney weight, therefore; the value does not represent a true value of right kidney GFR in the control animals. By using this method to estimate right kidney GFR in the control animals, a value was obtained so that a general comparison between the control and UNX groups could be made.

In the study by Moore et al., the GFR of 85-100 day old fetal lambs 72 hours post-UNX was 1.43 ± 0.11 mls/min in the control group and 1.53 ± 0.10 mls/min in the nephrectomy group (Moore et al., 1979). Both of these values are less than the measured

GFR in mls/min in our study for groups at 24 and 72 hours post-UNX. The differences in the results in the two studies can be attributed to experimental design differences. In the study by Moore et al. the fetal lambs studied were of a much younger age than ours (Moore et al., 1979). Nephrogenesis is completed by day 130 of gestation in the fetal lamb (Robillard et al., 1981). Prior to this time, the addition of new nephron units may be an important determinant of GFR, so at a gestational age prior to complete nephrogenesis, it may be expected that GFR would be less than after nephrogenesis is complete. In the fetal human, it has been shown that upon completion of nephrogenesis, RBF increases leading to an abrupt increase in GFR (Arant, 1978). Since we studied fetal lambs greater than 130 days gestation, any increase in GFR following UNX would be a reflection of an adaptive response in the functioning nephrons, and not the addition of new nephron units. Additionally, the renal function studies done by Moore et al. were performed while the ewe and fetus were under general anesthesia making comparison with the unanesthetized fetus difficult (Moore et al., 1979).

Our measured values for GFR in the control animals compares well with established values for GFR in the fetal lamb greater than 130 days gestation. Published values for GFR in fetal sheep greater than 130 days gestation are in range of 3.94 ± 0.4 mls/min. (Lumbers, 1983).

Many studies in the immature and adult animal measuring GFR in the acute phase following nephrectomy have demonstrated an absolute increase in GFR in mls/min, mls/min/kg and mls/min/gm kidney weight. In the late term fetal sheep, we were not able to demonstrate an increase in GFR 24 or 72 hours post-UNX except when comparing

remaining functional renal mass in the UNX group with single kidney GFR in the control animals. This demonstrates that the fetal kidney can produce a compensatory response to UNX in-utero albeit at a reduced magnitude when compared with the postnatal animal. If the more chronic response to UNX in the fetus were studied, then perhaps one would be able to measure a greater increase in GFR. However, because the placenta plays a major role in fluid and electrolyte homeostasis, the functional response to UNX in-utero may never be as complete as it is in the postnatal animal.

Fractional Clearance of Urinary Electrolytes

Fractional clearance of urinary electrolytes is a calculated value that represents the renal tubular ability to reabsorb or clear that substance, and reflects the rate at which a substance is removed from the blood into the urine. As was mentioned in the review of the literature, it is expected in the acute phase following UNX that fractional clearance of electrolytes will increase. This increase occurs because following UNX, hyperfiltration is not established in the immediate period, therefore; fractional clearance of electrolytes (especially sodium) per nephron must increase if intake is held constant in order to maintain balance (Hayslett, 1979). In our study, 24 hours post-UNX fractional clearance of sodium, potassium, chloride, and phosphate were all increased demonstrating glomerulotubular imbalance. None of the increases were statistically significant most likely due to the great variability in studying fetal individuals. This large variability was reflected in the SEM (see Tables 5 and 6).

In contrast, by 72 hours post-UNX, the fractional clearance of urinary electrolytes was greatly reduced compared with the 24 hour UNX values, and there was no significant

difference between the control and UNX groups. Other reports in the literature documenting changes in fractional clearance of electrolytes following UNX in the rat report that it takes two to four weeks for fluid reabsorption to be similar to control animals (Hayslett et al., 1968; Shirley and Walter, 1991). In this study, it appears as if glomerulotubular balance is restored to the normal fetal rate by 72 hours post-UNX as the clearance values were greatly reduced rate comparable to control animals. It is known in the adult rat that proximal tubular adaptive changes to UNX are completed by five days (Dicker and Walter, 1991). This process may be accelerated in the fetus and account for the rapid adjustment in clearance of electrolytes. The compensatory changes that occur in tubular function in the fetus following UNX would require micropuncture techniques to most fully document the response.

The only reported change in excretion in the fetus post-UNX measures phosphate excretion in the fetal lamb (Moore et al., 1979). This study found a significant increase in phosphate excretion 72 hours post-UNX. These results are different from ours, again possibly due to the difference in fetal age and study conditions.

Urine Production

In our study, we found no statistical difference in urine production 24 or 72 hours post-UNX. In the study by Moore et al. studying fetal lambs 72 hours post-UNX, a statistically significant increase in urine production was found (Moore et al., 1979). An increase in urine production would be expected in the acute phase following UNX because sodium excretion is increased causing water to follow. This increase in water loss is directly linked to inhibition of sodium reabsorption (Allison et al., 1973; Dirks et al., 1973; Shirley and Walter, 1991). The lack of finding increased urine production in the acute phase

following UNX in our study may be related to the hydration status of the ewe and/or fetus, or changes in systemic blood pressure or intra-renal blood flow. However, if the control urine flow rate (Tables 7 and 8) is halved providing comparison of control one-kidney urine flow rate to remnant kidney urine flow rate, a 54% and 24% increase in urine flow rate 24 and 72 hours post-UNX is revealed, respectively. Halving of the two-kidney control urine flow rate to obtain a one-kidney urine flow rate provides only an approximation of one kidney urine flow rate as ureteral catheterization would be necessary to obtain accurate one-kidney urine flow rates. By using this method to approximate control one-kidney urine flow rate, it is apparent that the remnant kidney is producing more urine than the single control kidney.

To summarize, it appears that the late gestational fetal sheep can functionally adapt to an acute reduction in renal mass. Adaptation in this situation is demonstrated by an increase in GFR per gram kidney weight and by rapid restoration of glomerulotubular balance.

Table 1. Glomerular Filtration Rate (GFR) 24 Hours Post-Uninephrectomy.

Group	n	BW ^a	GFR (mls/min)	GFR (mls/min/kg)	GFR (mls/min/gm total kidney wt)
Control	9	3.93 ± 0.42	3.84 ± 0.71	0.92 ± 0.09	0.17 ± 0.02
Nephrectomy	11	3.82 ± 0.28	2.23 ± 0.27*	0.57 ± 0.04*	0.20 ± 0.02

* P ≤ 0.05 versus control group

^a BW = body weight in kilograms

Table 2. 24 Hour Right Kidney Glomerular Filtration Rate (GFR).

Group	n	GFR (mls/min/gm right kidney wt)
Control	9	0.08 ± 0.01
Nephrectomy	11	0.20 ± 0.02*

* P ≤ 0.05 versus control group

Table 3. Glomerular Filtration Rate (GFR) 72 Hours Post-Uninephrectomy.

Group	n	BW ^a	GFR (mls/min)	GFR (mls/min/kg)	GFR (mls/min/gm total kidney wt)
Control	6	3.33 ± 0.32	3.45 ± 0.47	1.03 ± 0.07	0.16 ± 0.01
Nephrectomy	5	3.35 ± 0.43	1.98 ± 0.49*	0.59 ± 0.07*	0.17 ± 0.03

^a BW = body weight in kilograms

* P ≤ 0.05 versus control group

Table 4. 72 Hour Right Kidney Glomerular Filtration Rate (GFR).

Group	n	GFR (mls/min/gm right kidney wt)
Control	6	0.08 ± 0.01
Nephrectomy	5	0.17 ± 0.03*

* P ≤ 0.05 versus control group

Table 5. Fractional Clearance of Urinary Electrolytes 24 Hours Post-Uninephrectomy.

Group	n	% Fe Na	% Fe K	% Fe Cl	% Fe PO ₄	Urinary Protein (mg/ml)
Control	9	8.38 ± 1.95	33 ± 6.52	8.89 ± 2.2	27.3 ± 7.48	0.38 ± 0.13
Nephrectomy	11	12.01 ± 7.17	80 ± 20.92	16.38 ± 2.97	28.4 ± 11.46	0.48 ± 0.14

Table 6. Fractional Clearance of Urinary Electrolytes 72 Hours Post-Uninephrectomy.

Group	n	% Fe Na	% Fe K	% Fe Cl	% Fe PO ₄	Urinary Protein (mg/ml)
Control	6	7.16 ± 2.49	41.1 ± 12.42	7.48 ± 3.01	20.8 ± 11.9	0.29 ± 0.08
Nephrectomy	5	5.39 ± 1.21	21.2 ± 9.88	5.33 ± 2.03	25.6 ± 17.9	0.44 ± 0.21

Table 7. Urine Flow Rate 24 Hours Post-Uninephrectomy.

Group	n	Urine Flow Rate (mls/20 min collection period)
Control	9	11.77 ± 1.88
Nephrectomy	11	9.06 ± 1.45

Table 8. Urine Flow Rate 72 Hours Post-Uninephrectomy.

Group	n	Urine Flow Rate (mls/20 min collection period)
Control	6	11.34 ± 2.65
Nephrectomy	5	7.03 ± 1.79

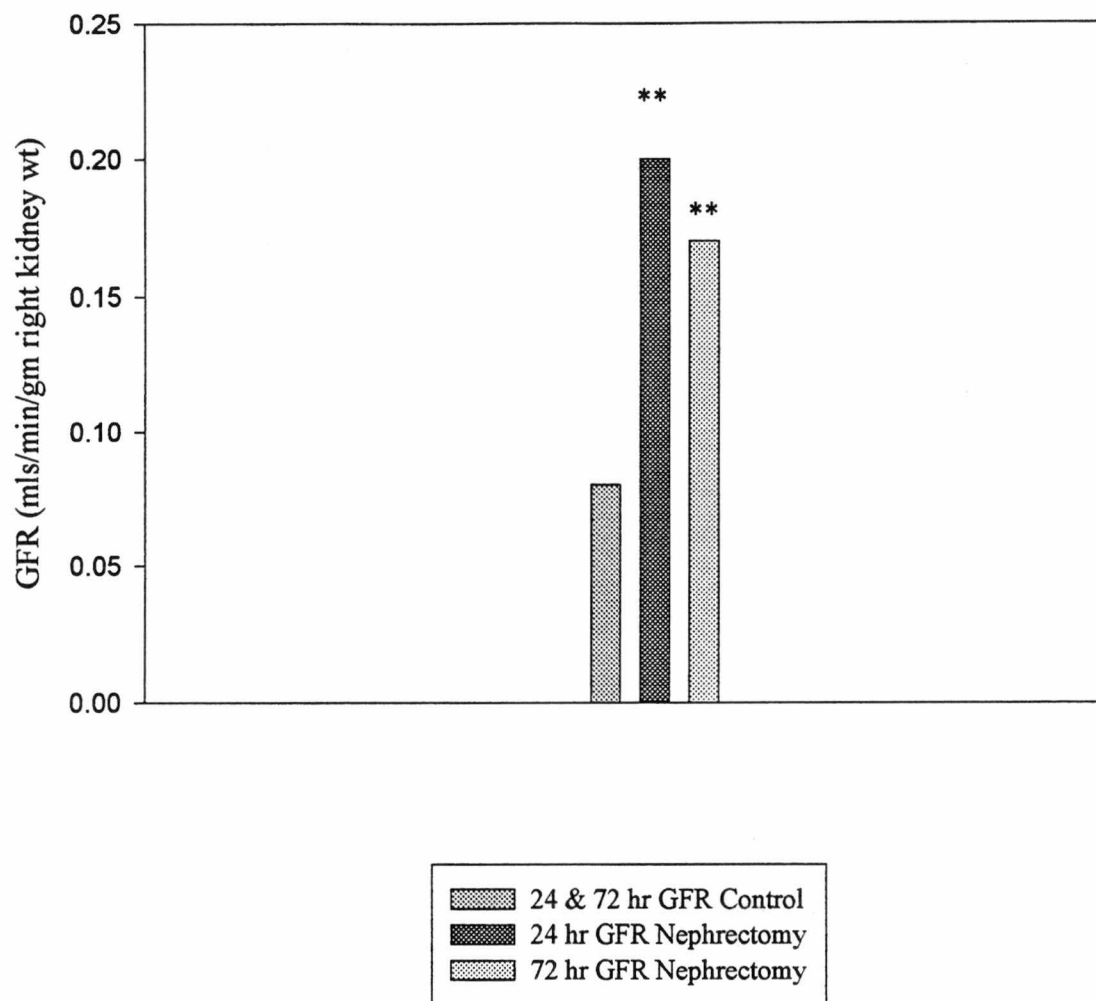


Figure 1. Right Kidney Glomerular Filtration Rate 24 and 72 Hours Post-Uninephrectomy

** = $P \leq 0.05$ versus control group

LITERATURE CITED

- Allison ME, Lipham EM, Lassiter WE et al. The acutely reduced kidney. *Kidney Int.* 3:354-363, 1973.
- Arant BS Jr. Developmental patterns of renal functional maturation compared in the human neonate. *J Pediatr.* 92:705-, 1978.
- Aschinberg LC, Koskimies O, Bernstein J et al. The influence of age on the response to renal parenchymal loss. *Yale J Biol Med.* 51:341-345, 1978.
- Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem.* 72:248-254, 1976.
- Buerkert J, Martin D, Prasad J et al. Response of deep nephrons and the terminal collecting duct to a reduction in renal mass. *Am J Physiol.* 236:F454-F464, 1979.
- Carriere S, Gagnan-Brunette M. Compensatory renal hypertrophy in dogs: Single nephron glomerular filtration rate. *Can J Physiol Pharmacol.* 55:105-110, 1977.
- Chen PS, Toribara TY, Warner H. Microdetermination of phosphorus. *Anal Chem.* 28:1756-1758, 1956.
- Chevalier RL. Functional adaptation to reduced renal mass in early development. *Am J Physiol.* 242:F190-F196, 1982.
- Chevalier RL. Reduced renal mass in early postnatal development: Glomerular dynamics in the guinea pig. *Biol Neonate.* 44:158-165, 1983.
- Chevalier RL. The response to nephron loss in early development. In: *Fetal and Neonatal Physiology*. Polin RA and Fox WW eds. WB Saunders Company, Philadelphia. 2:1264-1, 1992.
- Chevalier RL, Kaiser DL. Effects of acute uninephrectomy and age on renal blood flow autoregulation in the rat. *Am J Physiol.* 249:F672-F679, 1985.
- Diezi J, Michoud P, Grandchamp A et al. Effects of nephrectomy on renal salt and water transport in the remaining kidney. *Kidney Int.* 10:450-462, 1976.
- Diezi J, Michoud-Hausel P, Nicolas-Buxcel N. Studies on possible mechanisms of early functional compensatory adaptation in the remaining kidney. *Yale J Biol Med.* 51:265-270, 1978.

Dirks JH, Wong NLM, Evanson RL. Reduction in proximal tubular fluid reabsorption after clamping the contralateral kidney in the dog. *Clin Res.* 19:528, 1973.

Dirks JH, Wong NL. Acute functional adaptation to nephron loss: Micropuncture studies. *Yale J Biol Med.* 51:255-263, 1978.

Galla JH, Klein-Robbenhaar T, Hayslett JP. Influence of age on compensatory response in growth and function to unilateral nephrectomy. *Yale J Biol Med.* 47:218-226, 1974.

Hayslett JP, Kashgarian M, Epstein FH. Functional correlates of compensatory renal hypertrophy. *J Clin Invest.* 47:744-799, 1968.

Hayslett JP. Functional adaptation to reduction in renal mass. *Physiol Rev.* 59:137-164, 1979.

Hill KJ, Lumbers ER. Renal function in adult and fetal sheep. *J Dev Physiol.* 10:149-159, 1988.

Hayslett JP, Kashgarian M, Epstein FH. Functional correlates of compensatory renal hypertrophy. *J Clin Invest.* 47:774-782, 1968.

Humphreys MH, Ayus JC. Role of hemodynamic changes in the increased cation excretion after acute unilateral nephrectomy in the anesthetized dog. *J Clin Invest.* 61:590-596, 1978.

Kaufman JM, Siegel NJ, Hayslett JP. Functional and hemodynamic adaptation to progressive renal ablation. *Circ Res.* 36:286-293, 1975.

Krohn AG, Peng BB, Antell HI et al. Compensatory renal hypertrophy: The role of immediate vascular changes in its production. *J Urol.* 103:564-568, 1970.

Lumbers ER. A brief review of fetal renal function. *J Dev Physiol.* 6:1-10, 1983.

Lumbers ER, Hill KJ, Bennett VJ. Proximal and distal tubular activity in chronically catheterized fetal sheep compared with the adult. *Can J Physiol Pharmacol.* 66:697-702, 1988.

Moore ES, deLeon LB, Weiss LS et al. Compensatory renal hypertrophy in fetal lambs. *Pediatr Res.* 13:1125-1128, 1979.

Mulroney SE, Lumpkin MD, Haramati A. Antagonist to GH-releasing factor inhibits growth and renal Pi reabsorption in immature rats. *Am J Physiol.* 257:F29-F34, 1989.

Peters G. Compensatory adaptation of renal functions in the unanesthetized rat. *Am J Physiol.* 205:1042-1048, 1963.

Potter DE, Leumann EP, Sakai T et al. Early responses of glomerular filtration rate to unilateral nephrectomy. *Kidney Int.* 5:131-136, 1974.

Provoost AP, Molenaar JC. Changes in the glomerular filtration rate after unilateral nephrectomy in rats. *Pflugers Arch.* 385:161-165, 1980.

Ribstein J, Humphreys MH. Renal nerves and cation excretion after acute reduction in functioning renal mass in the rat. *Am J Physiol.* 246:F260-F265, 1984.

Robillard JE, Sessions C, Kennedy RL et al. Interrelationship between glomerular filtration rate and renal transport of sodium and chloride during fetal life. *Am J Obstet Gynecol.* 128:727-734, 1977.

Robillard JE, Ramberg E, Sessions C et al. Role of aldosterone on renal sodium and potassium excretion during fetal life and the newborn period. *Dev Pharmacol Ther.* 1:201-216, 1980.

Robillard JE, Weismann DN, Herin P. Ontogeny of single glomerular perfusion rate in fetal and newborn lambs. *Pediatr Res.* 15:1248-1255, 1981.

Robillard JE, Smith FG, Smith FG. Developmental aspects of renal function during fetal life. In: *Pediatric Kidney Disease*. Edelmann CM ed. Little, Brown and Company, Boston. 3-18, 1992.

Shirley DG, Walter SJ. Acute and chronic changes in renal function following unilateral nephrectomy. *Kidney Int.* 40:62-68, 1991.

Spitzer A, Brandis M. Functional and morphologic maturation of the superficial nephrons: Relationship to the total kidney function. *J Clin Invest.* 53:279-287, 1974.

Spitzer A, Edelmann CM Jr. Maturation changes in pressure gradients for glomerular filtration. *Am J Physiol.* 211:1431-1435, 1971.

PART 3

THE EFFECT OF UNILATERAL NEPHRECTOMY ON

COMPENSATORY RENAL GROWTH

ABSTRACT

It has long been known that following a reduction in renal mass, the remaining renal tissue undergoes compensatory renal growth (CRG). In response to uninephrectomy (UNX), there are age-related differences in the type of CRG. Adult animals primarily undergo hypertrophy and young or immature animals primarily undergo hyperplasia. Studies examining CRG in the fetus are conflicting. Some investigators have found no evidence of CRG following UNX and others have identified a significant growth response. To address this issue, a study was performed in the late gestational ovine fetus to examine the magnitude and type of CRG following left UNX. No significant increase in kidney weight was found 24 or 72 hours post-UNX versus sham operated age-matched controls. However, 72 hours post-UNX, there was a trend towards an increased weight in the remnant kidney. The weight of the right remnant kidney had increased in total weight by 8.5% over the normal growth rate 72 hours post-UNX. Protein and DNA content of the kidney was used as a measure of hypertrophy and hyperplasia, respectively. Neither of these parameters were increased over sham operated animals. But, at 72 hours post-UNX, total remnant kidney DNA content had increased 40% and total remnant kidney protein content had increased 51% compared to the extant kidney. It appears that the late gestational ovine fetus does not demonstrate rapid CRG, but there is an indication of hypertrophy and hyperplasia in the remnant kidney by 72 hours post-UNX. This is in sharp contrast to postnatal animals in which significant CRG can be demonstrated following UNX within 24 hours. It may be that the late gestational fetus needs a more chronic time course to undergo CRG in response to UNX.

REVIEW OF THE LITERATURE

Overview of Compensatory Renal Growth

It has been known for centuries that removal, damage, or absence of one kidney results in adaptive or compensatory renal growth (CRG) of the remaining kidney (Fine, 1986). Compensatory renal growth can occur in two forms: hypertrophy and/or hyperplasia. Hypertrophy is defined as an increase in cell size and experimentally manifests as an increase in tissue protein per DNA, protein per cell, or RNA:DNA ratio (Fine, 1986). With hypertrophy, all of the component parts of the nephron are enlarged, but the proximal tubule enlarges out of proportion to the rest of the nephron segments (Oliver, 1944; Fine, 1986; Fine and Norman, 1989). On the ultrastructural level, it has been shown that the basolateral membrane of the proximal tubule hypertrophies to a greater extent than the luminal membrane (Salehmoghaddam et al., 1985). The increased size of the proximal tubule is associated with increased reabsorption of solutes in order to maintain glomerulotubular balance.

Hyperplasia is defined as an increase in cell number and experimentally manifests as an increase in DNA content (Fine, 1986). With renal hyperplasia, no new nephron formation results, so the increase in DNA represents an increase in the number of cells per existing nephrons (Larsson et al., 1980).

Changes in RNA, protein, and DNA can occur within hours following a reduction in renal mass and increases in total renal weight have been reported as early as 24 hours. Increases in RNA can occur as early as 4 hours post reduction in renal mass and is largely attributed to an increased production in ribosomal RNA synthesis (Meyer et al., 1996).

However, decreased degradation of RNA may also play a role. Increases in protein content occur following increases in RNA and increases in DNA content generally occur last (Meyer et al., 1996). Increased DNA synthesis is not thought to occur before 9 to 18 hours following a reduction in renal mass (Meyer et al., 1996). Although the compensatory response begins very quickly following a reduction in renal mass, the total increase in mass is not complete for up to four weeks (Malt, 1969; Fine, 1986; Meyer, 1996).

Age-related Differences in Compensatory Renal Growth

Unilateral nephrectomy (UNX) is an experimental method that is commonly used to initiate the CRG response. The manner in which the adult and immature animal responds to this procedure is different. In the adult, approximately 80% of CRG is due to hypertrophy of primarily the proximal tubule (Johnson and Vera Roman, 1966; Johnson, 1969). Increases in both RNA and protein, indicating hypertrophy, have been demonstrated within one hour of UNX (Johnson and Vera Roman, 1966). The remaining 20% of the response is due to hyperplasia which is demonstrated by an increase in DNA by approximately 18 hours post-UNX (Johnson and Vera Roman, 1966; Fine, 1986). The increase in DNA is transient with the maximum reached at 48 hours post-UNX (Johnson and Vera Roman, 1966). Thus, in the adult animal, hypertrophy is the primary and predominant response to UNX implying the mitotic capacity is markedly reduced after renal cells reach terminal differentiation.

The response to UNX in the young animal is somewhat different. The young (neonatal, immature, or juvenile) animal responds to UNX predominately by hyperplasia with the overall response to UNX greater than the adult (Dicker and Shirley, 1973; Hayslett, 1983; Celsi et al., 1986). Comparison of the response of adult and newborn rats to UNX

found adults increased their remaining kidney weight by 30-40%, but newborn rats were able to increase their kidney weight by 70% (MacKay et al., 1932). At one time it was thought that young animals had the capacity to add glomeruli in response to UNX, but this has been disproved with the advent of more sophisticated glomerular counting techniques (Kaufman et al., 1975; Chevalier, 1982). It has also been proven that CRG continues for a longer period in the young animal (Kaufman et al., 1975; Celsi et al., 1986). Celsi et al. demonstrated that following UNX, CRG in adult rats was completed within three weeks, but in young rats the process continued for 8-10 weeks (Celsi et al., 1986).

The reason for the age-related difference to UNX is still a matter of debate. However, in all individuals, CRG is proportional to the magnitude of nephron loss, but the degree of compensation is always greater in the young individual (Fine, 1986; Chevalier, 1992). Also, it has been found that over time, the extant kidney increases in mass by approximately 50% and that remaining glomeruli increase in volume by 50% (Hostetter, 1995; Meyer et al., 1996). The question as to why CRG is primarily due to hyperplasia and is more complete in the young still remains. The triggering mechanism(s) for CRG have thus far eluded identification as well. This subject will be addressed in Part 4.

Fetal Compensatory Renal Growth

There are few studies on CRG in the fetus. Goss and Walker studied the effects of UNX on fetal rats at day 19 of gestation. They found no difference in kidney weight versus sham operated animals at 24 hours post-UNX. By 48 hours, there was a significant 8.6% increase in kidney weight of UNX animals versus sham operated animals. By 72 hours post-UNX the increase over sham operated animals was 4.9% and was no longer significant (Goss

and Walker, 1971). However, in a study by Rollason, no evidence of CRG was found in fetal rates uninephrectomized on day 19 of gestation either 24 or 48 hours later (Rollason, 1969).

There are two studies in the literature studying CRG in the fetal lamb. In a study by Moore et al. fetal lambs between day 85-100 of gestation underwent UNX and were studied 72 hours later. They found statistically significant increases in weight of the renoprival kidney and in renal cortical RNA and DNA (Moore et al., 1979). This study demonstrated that the increase in weight of the renoprival kidney was due to both hypertrophy and hyperplasia.

In a study by Peters et al., compensatory renal growth following unilateral ureteral obstruction was examined in fetal sheep. Surgical perturbation was performed on day 60 of gestation with studies performed subsequently on day 75, 95, and 135 of gestation. Their results indicated that the growth rate of the contralateral kidney was accelerated compared with that of the control animal until 95 days of gestation. After that time, the growth rate was not different from the controls. The growth response was hyperplastic in nature as DNA concentration increased in parallel with the increase in kidney weight. No increase in RNA or protein was measured. The average weight of both kidneys in the control and operated groups was relatively constant at each time period studied. The hyperplastic response was due only to addition of new cells per existing nephron as the total number of nephrons in the control and operated groups was the same (Peters et al., 1993).

In a series of 20 human fetal autopsies with unilateral renal agenesis, Harteshorne et al. identified CRG (Harteshorne, 1990). Laufer and Griscom used excretory urography to

detect CRG in human newborns with unilateral nonfunctioning multicystic kidney disease. Using this method, they were unable to detect any evidence of CRG (Laufer and Griscom, 1971).

Because the placenta performs most of the homeostatic functions performed by the kidney in postnatal life, it is questionable why CRG would occur to a significant degree in the fetus. To address this question, several investigators have performed maternal nephrectomies and studied the fetal response. Rollason performed bilateral maternal nephrectomies in the rat on day 18½ of gestation and found an elevated mitotic index in the nephrogenic zones of fetal kidneys and that fetal proximal tubule cell differentiation became more rapid (Rollason, 1961). However, Goss found no increase in mitotic index in the cortical areas of fetal rat kidneys from mothers uninephrectomized on day 19 of pregnancy (Goss, 1963). In a study by Malt and Lemaitre measuring nucleic acids in the fetal kidneys of mice from mothers uninephrectomized on day 17 of pregnancy, no increase in RNA/DNA could be found in the fetal kidneys (Malt and Lemaitre, 1969).

Thus the occurrence of CRG in the fetus is still controversial. To examine the possibility of CRG in response to an acute reduction in renal mass in-utero, UNX was performed in the late gestation ovine fetus. Parameters examined as outcomes to address the general question of the occurrence of in-utero CRG included growth rate of the contralateral kidney as indicated by change in weight, and DNA and protein content.

MATERIALS AND METHODS

Animal Instrumentation

All animals were subjected to health screening tests and acclimated as per Materials and Methods in Part 2. Surgical instrumentation and manipulation were also performed as per Materials and Methods in Part 2.

Sample Collection

At the time of nephrectomy and at the end of the physiologic study period, kidney tissues were obtained for DNA and protein analysis. At the time of harvest, the tissues were placed in individual plastic cups containing chilled 0.9% NaCl and placed on ice.

Analytical Methods

Following tissue removal, the entire kidney was weighed. A 0.5 gm full thickness piece of kidney tissue was obtained for DNA and protein analysis. The remainder of the kidney tissue was snap frozen in liquid nitrogen for future analysis.

The 0.5 gm slice of kidney tissue was homogenized in 10 mM Hepes in 1 mM EGTA (pH 7.4) using an overhead stirrer with Teflon homogenizer (S/N: 0653 Wheaton Company, Millville, NJ). Total kidney DNA concentration in the homogenate was determined by the diphenylamine spectrophotometric method (Richards, 1974). Total kidney protein concentration in the homogenate was determined by the Lowry spectrophotometric method (Lowry et al., 1951). The amount of kidney homogenate used in the DNA assay was 20 μ l, and the amount of kidney homogenate used in the protein assay was 40 μ l. The DNA assay was read on a spectrophotometer at 600 nm, and the protein assay at 540 nm (Spectronic 610, Milton Roy Company, Rochester, NY).

Statistical Analysis

Significant differences between the means of the control (sham operated) and nephrectomized fetal lambs were determined by Student's *t* test. Significant differences between the means within groups was determined by paired Student's *t* test. Significance was determined at the $P \leq 0.05$ level. SigmaStat™ statistical software was used for all statistical analysis (Jandel Scientific, SanRafel, CA).

RESULTS

Normal Kidney Growth Rate

24 hours

Comparison of the left and right kidneys of the control group showed no significant difference between the means $\pm$ standard error of the mean (SEM) of the weights. Comparison of the left kidney of the nephrectomy group with the left kidney of the control group was used to establish the normal growth rate of the kidney with time. This comparison demonstrated a 20.8% increase in weight of the left kidney in 24 hours, representing the growth rate.

72 hours

Comparison of the left and right kidneys of the control group showed no significant difference between the means $\pm$ SEM of the weights. Comparison of the left kidney of the nephrectomy group with the left kidney of the control group was used to establish the normal growth rate of the kidney with time. This comparison demonstrated a 14.5% increase in weight of the left kidney in 72 hours, representing the growth rate.

Change in Kidney Weight

24 hours post-uninephrectomy

Table 1¹ shows the change in kidney weight 24 hours post-UNX. Values are expressed as mean $\pm$ SEM. Body weight in the control (sham operated) group was 4.25 ± 0.37 kg and weight in the nephrectomy group was 3.60 ± 0.27 kg. The difference in weights

¹ All Tables and Figures appear after the Discussion section.

between the two groups was not statistically different. The weight of the control left kidney was 11.60 ± 0.85 gms and the weight of the nephrectomy left kidney 9.60 ± 0.64 gms. The difference in the kidney weights was not statistically different. The weight of the control right kidney was 11.20 ± 0.85 gms and the weight of the nephrectomy right kidney 10.6 ± 0.62 gms. The difference in the kidney weights was not statistically different. The difference in weights of the nephrectomized left (9.60 ± 0.64 gms) and right (10.60 ± 0.62 gms) kidneys represented a 10.4% change and was statistically significant ($P \leq 0.05$). The total kidney weight to body weight ratio in the control group was $0.56 \pm 0.05\%$ and $0.30 \pm 0.01\%$ in the nephrectomy group and represented a significant difference ($P \leq 0.05$).

Table 2 shows the individual kidney weight to body weight ratios between the control and nephrectomy groups. The left kidney comparison between the control ($0.27 \pm 0.03\%$) and nephrectomy ($0.27 \pm 0.02\%$) groups was not significantly different. The right kidney comparison between the control ($0.26 \pm 0.04\%$) and nephrectomy ($0.29 \pm 0.03\%$) groups was not significantly different.

72 hours post uninephrectomy

Table 3 shows the change in kidney weight 72 hours post-UNX. Values are expressed as mean $\pm$ SEM. Body weight in the control group was 3.31 ± 0.27 kg and weight in the nephrectomy group was 3.40 ± 0.33 kg. The difference in weights between the two groups was not statistically different. The weight of the control left kidney was 10.24 ± 0.85 gms and the weight of the left nephrectomy kidney 8.94 ± 0.68 gms. The difference in kidney weights between the two groups was not statistically different. The weight of the control right kidney was 10.00 ± 0.89 gms and the weight of the nephrectomy right kidney $12.3 \pm$

1.43 gms, representing a 23% difference. The difference in weights between the two groups was not statistically different. Comparison of right kidney weights 72 hours post-UNX is shown in Figure 1. The difference in weight between the nephrectomized left (8.94 ± 0.68 gms) and right (12.3 ± 1.43 gms) kidneys represented a 37.5% increase and was statistically significant ($P \leq 0.05$). Comparison of nephrectomy group left and right kidney weights 72 hours post-UNX is shown in Figure 2. The total kidney weight to body weight ratio in the control group was $0.61 \pm 0.02\%$ and $0.40 \pm 0.04\%$ in the nephrectomy group and represented a significant difference ($P \leq 0.05$).

Table 4 shows the individual kidney weight to body weight ratios between the control and nephrectomy groups. The left kidney comparison between the control ($0.31 \pm 0.03\%$) and nephrectomy ($0.27 \pm 0.09\%$) groups was not significantly different. The right kidney comparison between the control ($0.30 \pm 0.03\%$) and nephrectomy ($0.36 \pm 0.12\%$) groups was not significantly different. However, the right kidney weight to body weight ratio in the remnant kidney increased 20% over control values and 33% over extant kidney values.

Change in Kidney DNA and Protein Content

24 hours post-uninephrectomy

Table 5 shows the change in kidney DNA and protein concentration 24 hours post-UNX. Values are expressed as mean $\pm$ SEM. Left kidney DNA content was $25769.1 \pm 1156.6 \mu\text{g/gm}$ kidney wt in the control group and was $25631.9 \pm 1099.4 \mu\text{g/gm}$ kidney weight in the nephrectomy group. The right kidney DNA content was $24165.5 \pm 1469.1 \mu\text{g/gm}$ kidney weight in the control group and was $22991.2 \pm 1181.6 \mu\text{g/gm}$ kidney weight in the nephrectomy group. The differences in DNA concentration between the control and

nephrectomy groups was not statistically different for the left or right kidneys. The left kidney protein content was 85262.7 ± 4655.9 mg/gm kidney weight in the control group and was 87036.0 ± 5322.3 mg/gm kidney weight in the nephrectomy group. The right kidney protein content was 87876.1 ± 6667.7 mg/gm kidney weight in the control group and was 81132.7 ± 2146.2 mg/gm kidney weight in the nephrectomy group. The differences in protein concentration between the control and nephrectomy groups was not statistically different for the left or right kidneys.

The control left kidney DNA to protein ratio was 0.31 ± 0.02 and the ratio in the nephrectomy group was 0.31 ± 0.03 . The control right kidney DNA to protein ratio was 0.29 ± 0.03 and the ratio in the nephrectomy group was 0.28 ± 0.02 . The differences in DNA to protein ratios between the control and nephrectomy was not statistically different.

Paired *t* testing of the means within groups for DNA and protein concentration and DNA to protein ratio did not show any significant differences.

Table 6 shows the change in total kidney DNA and protein content 24 hours post-UNX. Values are expressed as mean $\pm$ SEM. Total left kidney DNA content was 0.229 ± 0.013 gms in the control group and was 0.246 ± 0.011 gms in the nephrectomy group. Total right kidney DNA content was 0.271 ± 0.016 gms in the control group and was 0.243 ± 0.013 gms in the nephrectomy group. Differences in DNA content between the control and nephrectomy groups was not statistically significant for left or right kidneys.

Total left kidney protein content was 0.989 ± 0.054 gms in the control group and was 0.836 ± 0.051 gms in the nephrectomy group. Total right kidney protein content was 0.984 ± 0.075 gms in the control group and was 0.860 ± 0.023 gms in the nephrectomy group.

Differences in protein content between the control and nephrectomy groups was not statistically different for left or right kidneys. Paired *t* testing between the means within the groups for total DNA and protein content did not show any significant differences.

72 hours post-nephrectomy

Table 7 shows the change in kidney DNA and protein concentration 72 hours post-UNX. Values are expressed as mean $\pm$ SEM. The left kidney DNA content was 22497.9 ± 2296.7 $\mu\text{g/gm}$ kidney weight in the control group and was 20205.0 ± 1748.9 $\mu\text{g/gm}$ kidney weight in the nephrectomy group. The difference in DNA content between the control and nephrectomy left kidneys was not statistically different. The right kidney DNA content was 26293.6 ± 1415.0 $\mu\text{g/gm}$ kidney weight in the control group and was 20030.0 ± 2010.5 $\mu\text{g/gm}$ kidney weight in the nephrectomy group. The difference in DNA content between the right kidney in the control and nephrectomy groups was 31.3% and was statistically significant ($P \leq 0.05$).

The protein content in the left kidney was 84809.4 ± 4573.9 mg/gm kidney weight in the control group and was 72551.9 ± 6714.9 mg/gm kidney weight in the nephrectomy group. The protein content in the right kidney was 79030.5 ± 4573.9 mg/gm kidney weight in the control group and was 75090.9 ± 7860.2 mg/gm kidney weight in the nephrectomy group. The differences in protein concentration between the control and nephrectomy groups was not statistically different for the left or right kidneys.

The control left kidney DNA to protein ratio was 3.28 ± 0.04 and the ratio in the nephrectomy group was 0.29 ± 0.03 . The control right kidney DNA to protein ratio was 0.34

± 0.02 and the ratio in the nephrectomy group was 0.28 ± 0.04 . The difference in ratios between the groups was not statistically different for the left or right kidneys.

Paired *t* testing of the means within groups for DNA and protein concentration and DNA to protein ratio did not show any significant differences.

Table 8 shows the change in total kidney DNA and protein content 72 hours post-UNX. Values are expressed as mean $\pm$ SEM. Total left kidney DNA content was 0.231 ± 0.030 gms in the control group and was 0.171 ± 0.020 gms in the nephrectomy group. Total right kidney DNA content was 0.263 ± 0.025 gms in the control group and was 0.239 ± 0.025 in the nephrectomy group. Differences in total DNA between control and nephrectomy groups was not statistically significant for left or right kidneys. The 40% increase in DNA between the left and right kidneys of the nephrectomy group was statistically significant at the $P \leq 0.05$ level.

Total left kidney protein content was 0.866 ± 0.106 gms in the control group and was 0.637 ± 0.208 gms in the nephrectomy group. Total right kidney protein content was 0.788 ± 0.077 gms in the control group and was 0.963 ± 0.208 in the nephrectomy group. Differences in protein content between the control and nephrectomy groups was not statistically significant for left or right kidneys. There was a 51% increase in protein content between left and right kidneys in the nephrectomy group, but this increase was not statistically significant.

DISCUSSION

Normal Growth Rate

Before any statement regarding CRG in response to UNX in the fetus can be made, normal growth rates should be taken into account. During the fetal period, growth is maximal, therefore; change in growth of the remaining kidney following UNX should be viewed in light of the normal growth that is occurring. The magnitude of CRG in response to UNX should ideally should be made against age-matched controls that take normal growth rate into account. However, this is difficult to establish in the fetal sheep because there are multiple fetuses with different body and kidney weights as well as tremendous variability between animals. The more critical analysis of growth changes in response to UNX may be performed by comparing left and right kidneys in the nephrectomy group. This method of comparison is the standard that is used in the postnatal animal. For completeness sake, analysis of the data was made between control and nephrectomy groups, and within the nephrectomy group.

The normal growth rate in this study was established by comparing the difference in kidney weight between the nephrectomized left kidney and the left kidney of the control animal 24 or 72 hours later. By using this method, the normal growth rate was 20.8% in 24 hours and 14.5% in 72 hours. The latter value is similar to the 11% normal renal growth rate established by Moore et al. in 72 hours in the midgestational fetal sheep (Moore et al., 1979). However, their method of establishing control values was based on twin studies where a twin fetus was removed and then 72 hours later the other twin removed. By comparing the differences in kidney weights between the twins, normal growth rates were established. The

study of unilateral ureteral obstruction in fetal sheep by Peters et al. did not report normal renal growth rates, however, age-matched controls were used for comparison (Peters et al., 1993).

It is important to note that the difference in body weights between the groups was not significantly different at each of the two time periods studied. Since kidney weight is proportional to body weight, the difference in body weight between the age-matched controls and nephrectomy group cannot be different if a direct comparison between the groups is used to establish a change in renal growth.

Change in Kidney Weight

In the present study, no significant increase in kidney weight was found 24 or 72 hours following UNX when comparing control and nephrectomy groups. Although by 72 hours post-UNX the renoprival kidney had increased in total weight 8.5% over the normal growth rate, demonstrating a trend toward CRG. These results are different from those of Moore et al. in which a significant increase in weight in the renoprival kidney was found 72 hours post-UNX in the fetal sheep (Moore et al., 1979). Differences in experimental design could account for the disparity in results. The fetal sheep in the current study were late gestational age versus midgestational age in the study by Moore et al. This could be a significant difference as nephrogenesis is complete after day 130 in the fetal sheep (Robillard et al., 1981). All animals studied by Moore et al. were between 85-100 days of gestation, and all animals studied in the current protocol were greater than 130 days gestation. In the midgestational fetal sheep, the animal is on a steeper phase of their growth curve and active

nephrogenesis is occurring, therefore; the ability of the animal to respond to a reduction in renal mass may be greater at this time.

The study of Peters et al. using the model of unilateral ureteral obstruction in the fetal sheep also demonstrated an increase in kidney weight. In this study, animals underwent ureteral ligation at day 60 of gestation and were studied at day 75, 95, and 135 of gestation. The increase in kidney weight only occurred until day 95 as the kidney weight at day 135 was not different from the weight at day 95 (Peters et al., 1993). Again this may indicate that the fetal kidney responds maximally to a reduction in renal mass when the perturbation occurs before nephrogenesis is complete. Also, if the current study had evaluated the response to UNX over a protracted time course, a significant increase in weight of the renoprival kidney over control values may have been identified.

The kidney weight to body weight ratio in the 24 and 72 hour groups was significantly greater in the control groups. Because body weight between the control and nephrectomy groups for each of the two time periods studied post-UNX was not different, and because kidney weight did not increase significantly during these two time periods, this ratio essentially reflects a halving of the total kidney weight in the nephrectomy groups. It is not surprising then that the kidney weight to body weight ratio in the control group would be significantly greater than in the nephrectomy group. However, the individual right kidney weight to body weight ratio, representing the proportion of the total body weight that is due to right kidney weight, increased 20% over control values and 33% over extant values 72 hours post-UNX. The 33% increase in the remnant kidney over the extant kidney was

statistically significant, and the increase in remnant right kidney weight to body weight demonstrated that CRG did occur.

Change in Kidney DNA and Protein Content

In the present study, no change in kidney DNA, protein, or DNA to protein ratio occurred 24 hours post-UNX when compared with age-matched controls. At 72 hours post-UNX, no changes occurred in kidney concentrations of DNA or protein or the DNA to protein ratio except for a significant decrease in right kidney DNA in the nephrectomy group. The reason for this change is not clear, however the SEM in this group was somewhat large and represented a 10% variance versus the control group in which the SEM represented a 5% variance. Also, the great variation in fetal animals could account for this difference.

When total kidney DNA and protein content was examined, a 40% increase in DNA and a 51% increase in protein content was demonstrated in the remnant kidney compared with the extant kidney 72 hours post-UNX. Additionally, total right kidney protein content was 36% greater in the remnant kidney compared with the right control kidney. This indicates that hypertrophy predominates over hyperplasia, an unexpected finding in light of the predominant hyperplastic response found in response to UNX in the immature animal.

Even though there was a 51% increase in total right kidney protein content 72 hours post-UNX in the remnant kidney compared with the extant kidney, the increase was not statistically significant. This finding is most likely related to the large variability in total kidney protein content between animals in the right kidney nephrectomy group, and is demonstrated by the large SEM in this group (Table 8).

Unlike the study by Moore et al. in which renal cortical RNA and DNA were increased 72 hours post-UNX, this study did not demonstrate any changes in markers of hypertrophy or hyperplasia when compared with control animals. The difference in results between the 2 studies could be explained by the age of the animals studied. This study examined late gestational fetuses versus midgestational fetuses used by Moore et al., and they also measured DNA and RNA in renal cortex only, but in the present study, DNA and protein were measured in whole kidney (Moore et al., 1979).

It appears that the late term gestational fetal sheep responds differently to an acute reduction in renal mass compared with the midgestational fetal sheep and certainly very differently from the young and adult animal. It is possible that if the late term fetus was exposed to a reduction in renal mass over time, a CRG response might become more apparent. However, in the present study in the acute period following UNX, CRG was demonstrated by an increase in remnant kidney weight to body weight ratio, and by an increase in total remnant kidney DNA and protein content. A dramatic acute response to UNX may not occur in the late gestational fetal lamb possibly because the fetus cannot respond with rapid growth during this time of its development, or because the placenta protects the fetus from some unknown stimulus that triggers CRG.

Table 1. Change in Kidney Weight 24 Hours Post-Uninephrectomy.

Group	n	Left Kidney wt ^a	Right Kidney wt	BW ^b	Kidney wt : BW (%)
Control	9	11.60 ± 0.85	11.20 ± 0.85	4.25 ± 0.37	0.56 ± 0.05
Nephrectomy	18	9.60 ± 0.64	10.6 ± 0.62**	3.60 ± 0.27	0.30 ± 0.01*

^a Kidney weight in grams

^b Body weight in kilograms

* P ≤ 0.05 versus control group

** P ≤ 0.05 versus nephrectomy group

Table 2. Kidney Weight to Body Weight (%) 24 Hours Post-Uninephrectomy.

Group	n	Left Kidney wt : BW	Right Kidney wt : BW
Control	9	0.27 ± 0.03	0.26 ± 0.04
Nephrectomy	18	0.27 ± 0.02	0.29 ± 0.03

Table 3. Change in Kidney Weight 72 Hours Post-Uninephrectomy.

Group	n	Left Kidney Wt ^a	Right Kidney Wt	BW ^b	Kidney Wt : BW (%)
Control	7	10.24 ± 0.85	10.00 ± 0.89	3.31 ± 0.27	0.61 ± 0.02
Nephrectomy	8	8.94 ± 0.68	12.3 ± 1.43**	3.40 ± 0.33	0.40 ± 0.04*

^a Kidney weight in grams

^b Body weight in kilograms

* P ≤ 0.05 versus control group

** P ≤ 0.05 versus nephrectomy group

Table 4. Kidney Weight to Body Weight (%) 72 Hours Post-Uninephrectomy.

Group	n	Left Kidney wt : BW	Right Kidney wt : BW
Control	7	0.31 ± 0.03	0.30 ± 0.03
Nephrectomy	8	0.27 ± 0.09	0.36 ± 0.12**

** P ≤ 0.05 versus nephrectomy group

Table 5. Change in Kidney DNA and Protein Concentration 24 Hours Post-Uninephrectomy.

Group	n	Left Kidney DNA ^a	Right Kidney DNA	Left Kidney Protein ^b	Right Kidney Protein	Left Kidney DNA : Protein	Right Kidney DNA : Protein
Control	9	25769.1 ± 1156.6	24165.5 ± 1469.1	85262.7 ± 4655.9	87876.1 ± 6667.7	0.31 ± 0.02	0.29 ± 0.03
Nephrectomy	13	25631.9 ± 1099.4	22991.2 ± 1181.6	87036.0 ± 5322.3	81132.7 ± 2146.2	0.31 ± 0.03	0.28 ± 0.02

^a DNA = μ g DNA per gm kidney wt

^b Protein = mg protein per gm kidney wt

Table 6. Total Kidney DNA and Protein Concentration 24 Hours Post-Uninephrectomy.

Group	n	Left Kidney DNA (gms)	Right Kidney DNA (gms)	Left Kidney Protein (gms)	Right Kidney Protein (gms)
Control	9	0.229 ± 0.013	0.271 ± 0.016	0.989 ± 0.054	0.984 ± 0.075
Nephrectomy	13	0.246 ± 0.011	0.243 ± 0.013	0.836 ± 0.051	0.860 ± 0.023

Table 7. Change in Kidney DNA and Protein Concentration 72 Hours Post-Uninephrectomy.

Group	n	Left Kidney DNA ^a	Right Kidney DNA	Left Kidney Protein ^b	Right Kidney Protein	Left Kidney DNA : Protein	Right Kidney DNA : Protein
Control	7	22497.9 ± 2296.7	26293.6 ± 1415.0	84809.4 ± 6686.9	79030.5 ± 4573.9	0.28 ± 0.04	0.34 ± 0.02
Nephrectomy	7	20205.0 ± 1748.9	20030.0 ± 2010.5*	72551.9 ± 6714.9	75090.9 ± 7860.2	0.29 ± 0.03	0.28 ± 0.04

^a DNA = μ g DNA per gm kidney wt

^b Protein = mg protein per gm kidney wt

* P $\leq$ 0.05 versus control group

Table 8. Total Kidney DNA and Protein Concentration 72 Hours Post-Uninephrectomy.

Group	n	Left Kidney DNA (gms)	Right Kidney DNA (gms)	Left Kidney Protein (gms)	Right Kidney Protein (gms)
Control	7	0.231 ± 0.030	0.263 ± 0.025	0.866 ± 0.106	0.788 ± 0.077
Nephrectomy	7	0.171 ± 0.020	0.239 ± 0.025**	0.637 ± 0.058	0.963 ± 0.208

** P $\leq$ 0.05 versus nephrectomy group

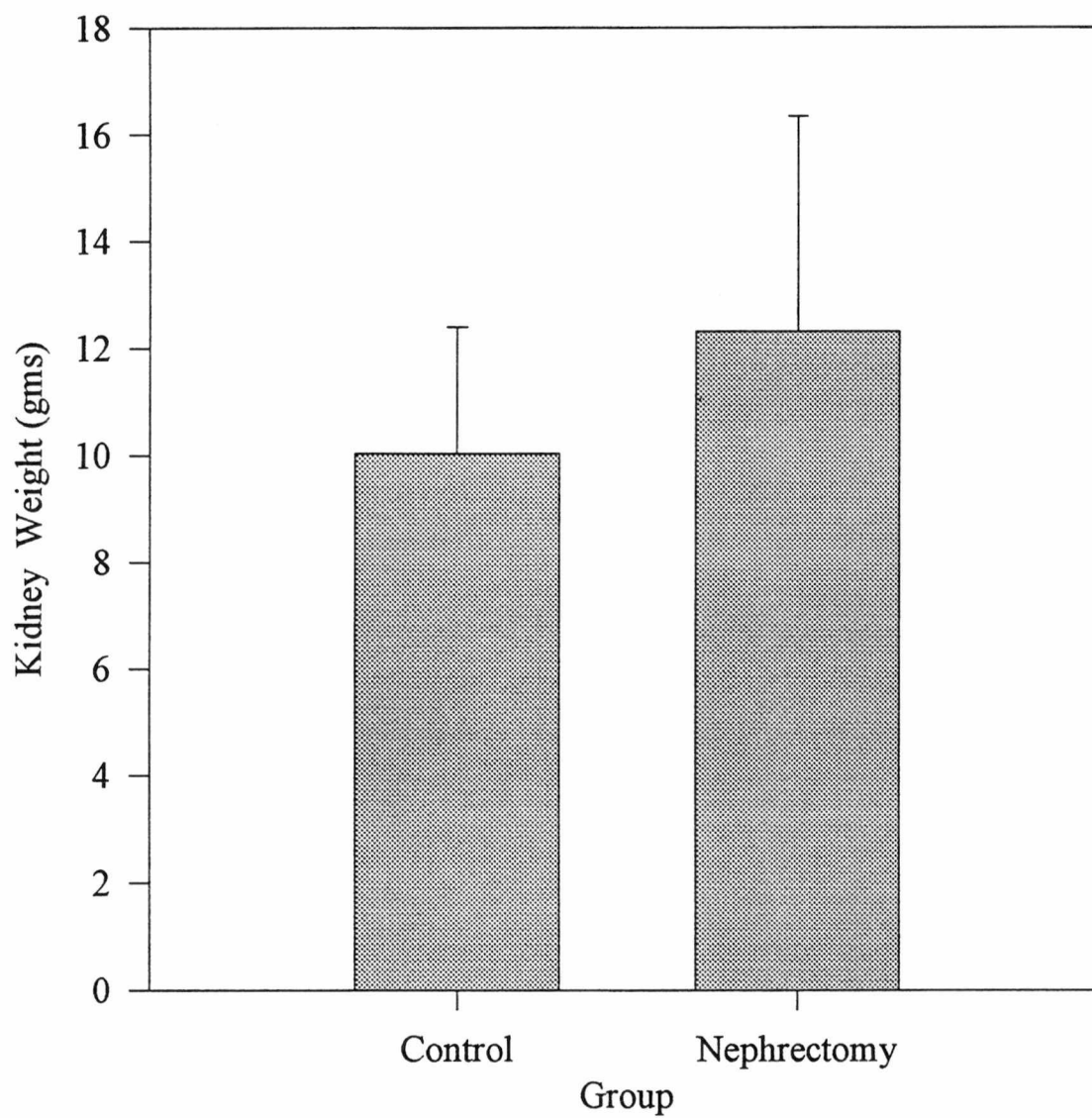


Figure 1. Comparison of Right Kidney Weights 72 Hours Post-Uninephrectomy

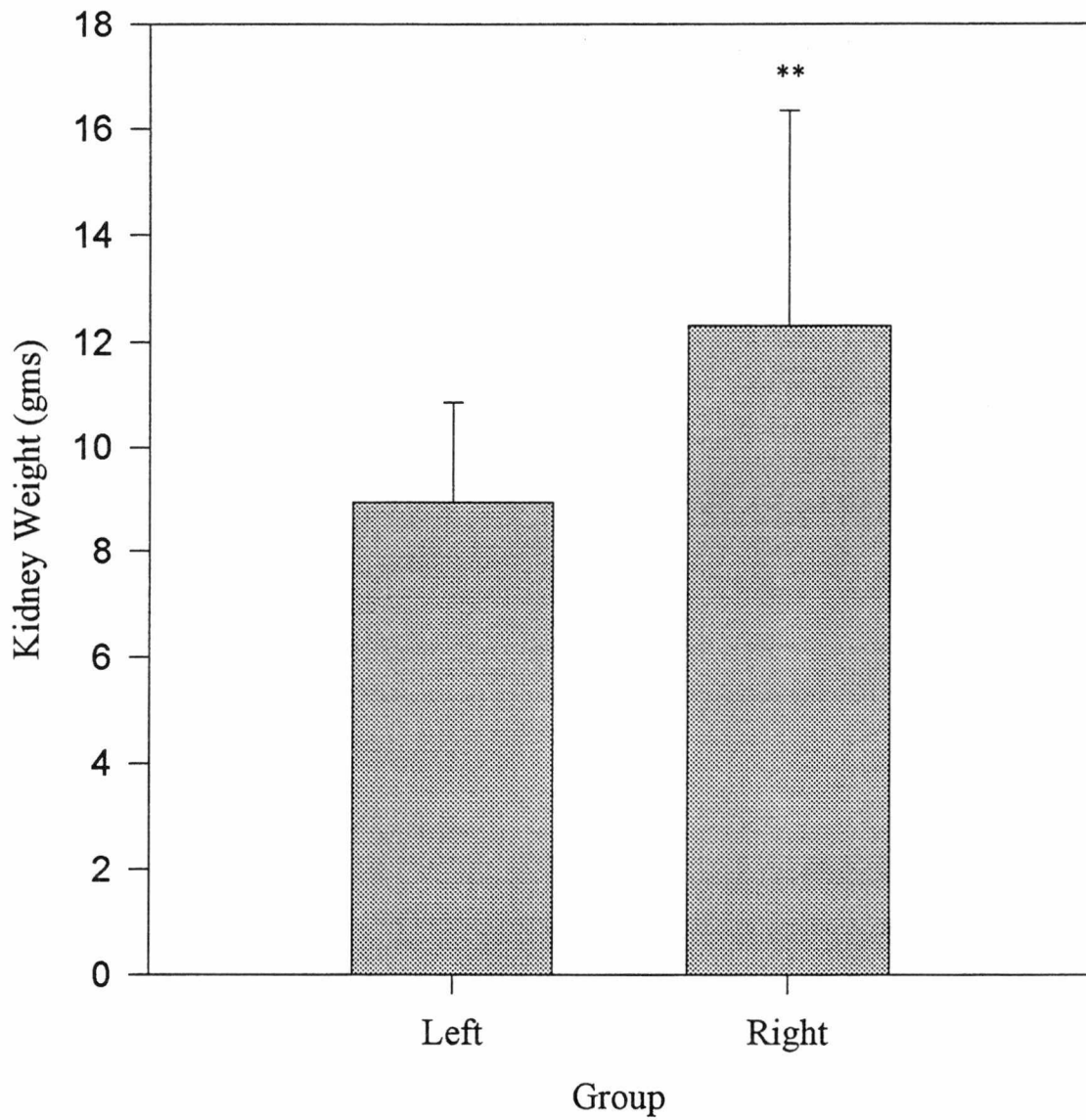


Figure 2. Comparison of Nephrectomy Group Left and Right Kidney Weights 72 Hours Post-Uninephrectomy

** = $P \leq 0.05$ versus control group

LITERATURE CITED

- Celsi G, Jakobsson B, Aperia A. Influence of age on compensatory renal growth in rats. *Pediatr Res.* 20:347-350, 1986.
- Chevalier RL. Glomerular number and perfusion during normal and compensatory renal growth in the guinea pig. *Pediatr Res.* 16:436-440, 1982.
- Dicker SE, Shirley DG. Compensatory renal growth after unilateral nephrectomy in the new-born rat. *J Physiol (Lond).* 228:193-202, 1973.
- Fine L. The biology of renal hypertrophy. *Kidney Int.* 29:619-634, 1986.
- Fine LG, Norman J. Cellular events in renal hypertrophy. *Annu Rev Physiol.* 51:19-32, 1989.
- Goss RJ. Effects of maternal nephrectomy on foetal kidneys. *Nature.* 198:1108-1109, 1963.
- Goss RJ, Walker MJ. Compensatory renal hypertrophy in fetal rats. *J Urol.* 106:360-362, 1971.
- Hartshorne N, Shepard T, Barr M Jr. Compensatory renal growth in human fetuses with unilateral renal agenesis. *Teratology.* 44:7-10, 1990.
- Hayslett JP. Effect of age on compensatory renal growth. *Kidney Int.* 23:599-602, 1983.
- Hostetter TH. Progression of renal disease and renal hypertrophy. *Annu Rev Physiol.* 57:263-278, 1995.
- Johnson HA, Vera Roman JM. Compensatory renal enlargement: Hypertrophy vs hyperplasia. *Amer J Pathol.* 49:1-13, 1966.
- Johnson HA. Cytoplasmic response to overwork. In: *Compensatory Renal Hypertrophy.* Nowinski WW and Goss RJ eds. Academic Press, New York. 9-25, 1969.
- Kaufman JM, Hardy R, Hayslett JP. Age-dependent characteristics of compensatory renal growth. *Kidney Int.* 8:21-26, 1975.
- Larsson L, Aperia A, Wilton P. Effect of normal development on compensatory renal growth. *Kidney Int.* 18:29-35, 1980.

- Lauffer I, Griscom NT. Compensatory renal hypertrophy. Absence in utero and development in early life. *Am J Roentgenol Radium Ther Nucl Med.* 113:464-467, 1971.
- Lowry OH, Rosebrough NJ, Farr HL et al. Protein measurement with the folin-phenol reagent. *J Biol Chem.* 193:265-275, 1951.
- MacKay EM, MacKay LL, Addis T. Degree of compensatory renal hypertrophy following unilateral nephrectomy; influence of age. *J Exp Med.* 56:255-265, 1932.
- Malt RA. Compensatory growth of the kidney. *N Engl J Med.* 280:1446-1459, 1969.
- Malt RA, Lemaitre DA. Nucleic acids in fetal kidney after maternal nephrectomy. *Pro Soc Exp Biol Med.* 130:539-542, 1969.
- Meyer TW, Baboolal K, Brenner BM. Nephron adaptation to renal injury. In: *The Kidney.* Brenner BM and Rector FC eds. WB Saunders Company, Philadelphia. 2:2011-2048, 1996.
- Moore ES, deLeon LB, Weiss LS et al. Compensatory renal hypertrophy in fetal lambs. *Pediatr Res.* 13:1125-1128, 1979.
- Oliver J. New directions in renal morphology: method, its results and its future. *Harvey Lect.* 40:102-155, 1945.
- Peters CA, Gaertner RC, Carr MC et al. Fetal compensatory renal growth due to unilateral ureteral obstruction. *J Urol.* 150:597-600, 1993.
- Richards GM. Modifications of the diphenylamine reaction giving increased sensitivity and simplicity in the estimation of DNA. *Anal Biochem.* 57:369-376, 1974.
- Robillard JE, Weismann DN, Herin P. Ontogeny of single glomerular perfusion rate in fetal and newborn lambs. *Pediatr Res.* 15:1248-1255, 1981.
- Rollason HD. Growth and differentiation of the fetal kidney following bilateral nephrectomy of the pregnant rat at 18½ days of gestation. *Anat Rec.* 141:183-193, 1961.
- Rollason HD. Mitotic activity in the fetal rat kidney following maternal and fetal nephrectomy. In: *Compensatory Renal Hypertrophy.* Nowinski WW and Goss RJ eds. Academic Press, New York. 61-68, 1969.
- Salehmoghaddam S, Bradley T, Mikhail N et al. Hypertrophy of basolateral Na-K pump activity in the proximal tubule of the remnant kidney. *Lab Invest.* 53:443-452, 1985.

PART 4

THE EFFECT OF UNILATERAL NEPHRECTOMY ON PLASMA GROWTH HORMONE CONCENTRATION

ABSTRACT

Removal of one kidney results in compensatory renal growth (CRG) of the remaining kidney. A potential initiating factor in CRG is growth hormone (GH). It has previously been determined that pulsatile GH secretion is increased following unilateral nephrectomy (UNX) in the adult male rat, but not in the immature rat. The possibility that pulsatile GH secretion may be altered in the fetus in response to UNX has not been examined. Therefore, the role of GH in CRG following UNX in the late gestational ovine fetus was examined. Plasma pulsatile GH secretion was measured by radioimmunoassay 24 and 72 hours post-UNX. Mean GH concentration 24 hours post-UNX (127.7 ± 13.6 ng/ml) was not statistically different from the control group (135.7 ± 14.7 ng/ml). Total GH secreted over the 5 hour study period 24 hours post-UNX (38163.4 ± 4013.3 ng/ml) was not statistically different from the control group (40893.2 ± 4491.6 ng/ml). The pulsatile nature of GH secretion 24 hours post-UNX was not different between the 2 groups and GH pulses occurred approximately every 2-2½ hours. Mean GH concentration 72 hours post-UNX (128.3 ± 17.8 ng/ml) was not statistically different from the control group (156.3 ± 17.9 ng/ml). Total GH secreted over the 5 hours study period 72 hours post-UNX (46815.2 ± 5321.0 ng/ml) was not statistically different from the control group (38463.8 ± 5387.1 ng/ml). The pulsatile nature of GH secretion 72 hours post-UNX was not different between the 2 groups and GH pulses occurred approximately every 2-2½ hours. Statistical significance was set at the $P \leq 0.05$ level. Therefore, it appears that GH is not an important mediator in the initial stages of the compensatory response to UNX in the late gestational ovine fetus.

REVIEW OF THE LITERATURE

That a reduction in renal mass results in the growth of the remaining renal tissue has been known for centuries. Although the growth response and the functional adaptation that accompany it have been described, the initiating step that triggers the compensatory process remains elusive (Fine, 1986; Meyer et al., 1996). It is also unknown if the initiating mechanism(s) of CRG occurs through local kidney factors or circulating factors. Several lines of thought have been advanced that attempt to account for the initiation of compensatory renal growth (CRG).

The Work Hypothesis

Because the kidneys are responsible for excreting the waste products of metabolism (urea), it was reasoned that renal enlargement following a reduction in renal mass was a simple response to an increased workload. This theory became known as the work hypothesis and was first introduced by Sacerdotti and later advanced by Addis (Sacerdotti, 1896; Addis, 1917). Because the idea seemed logical, it was accepted as fact for some time. However, studies administering a urea load failed to demonstrate CRG so the theory began to fall into disfavor (MacKay et al., 1932). An additional argument against the work hypothesis came from the model of uretero-peritoneostomy. In these studies, single nephron glomerular filtration rate and sodium reabsorption increased 50-100%, but CRG did not occur (Royce, 1963; Weinman et al., 1973). However, in these studies, it is possible that delivery of urine into the circulation may have an inhibitory effect on growth. More recent work has led to the conclusion that CRG is not triggered by an increase in kidney work linked to the reabsorption of solutes (Katz et al., 1978).

Circulating Stimuli

Because a reduction in renal mass leads to an organ-specific response, a search for a specific circulating stimulus for CRG has occurred. Several studies have been generated suggesting that a reduction in renal mass results in release of circulating factors (renotropic factors) that stimulate CRG. Increased renal DNA synthesis has been demonstrated following injection of plasma or serum from uninephrectomized (UNX) animals into normal animals (Lowenstein and Stern, 1963; Preuss and Glodin, 1976). Ogawa and Nowinski demonstrated an increased mitotic index in renal cells in culture in response to serum obtained from nephrectomized animals (Ogawa and Nowinski, 1958). This finding was substantiated by other investigators as well (Preuss and Glodin, 1976). However, there are other studies in the literature that were unable to demonstrate the presence of a serum or plasma renotropic factor (Reiter and McCreight, 1964; Johnson and Vera Roman, 1968).

Although no one factor has been demonstrated to exclusively induce renal hypertrophy and/or hyperplasia, several growth factors have been identified that induce renal growth. A list of some of the promising growth promoters includes: growth hormone (GH), insulin-like growth factor-I (IGF-I), epidermal growth factor (EGF), platelet-derived growth factor (PDGF), and hepatocyte growth factor (HGF) (Meyer et al., 1996). Other substances that are not classically thought of as growth factors but do have growth promoting properties include: thyroxine, cortisol, angiotensin II, arginine vasopressin, and prostaglandin E₂ (Meyer et al., 1996).

Growth Hormone

Secretory pattern

Recent evidence has been advanced for a role of GH in CRG. It has been known for a long time that GH is necessary for normal somatic growth and development. In most species including the sheep, GH is produced by acidophils and secreted from secretory granules in the adenohypophysis (Buonomo and Baile, 1990; Strobl and Thomas, 1994). Growth hormone release is stimulated by GH-releasing factor (GRF) which originates in the hypothalamus and traverses the hypophyseal portal system to reach the pituitary gland and stimulate GH secretion (Buonomo and Baile, 1990; Strobl and Thomas, 1994). Somatostatin (SRIF), also known as somatostatin-release inhibiting factor or GH-releasing inhibitory factor, also originates from the hypothalamus and traverses the hypophyseal portal system to inhibit GH release (Buonomo and Baile, 1990; Strobl and Thomas, 1994). Growth hormone is released in a pulsatile manner due to episodic secretion of GRF and SRIF. The episodic secretion of GRF and SRIF is due to feedback on these two releasing factors by GH at the hypothalamic level (Buonomo and Baile, 1990; Strobl and Thomas, 1994). Secretion of GH has positive effect on SRIF release and a negative effect on GRF release. In addition, other hormones, neurotransmitters, and peptides have effects on GRF and SRIF so that the release of GH is under control of a very complex feedback system. The complexities of GH release have been reviewed elsewhere (Buonomo and Baile, 1990). The pulsatile nature of GH release is responsible for its biological effects as constant or one time infusion of GH results in fewer pronounced effects versus pulsatile administration (Strobl, 1994).

Role in compensatory renal growth

Astarabadi and coworkers were the first to demonstrate that the anterior pituitary gland is required for CRG in response to UNX (Astarabadi and Essex, 1953; Astarabadi, 1963a, Astarabadi, 1963b). Other investigators found that CRG was most severely limited if nephrectomy was performed within 24-48 hours of hypophysectomy (Dicker et al., 1977). This study suggests that pituitary hormones may be important mediators of early CRG. Early on, GH was considered as the possible renotropic hormone of pituitary origin, but because the pulsatile nature of GH secretion was not known at the time of these studies, single time point determination of GH post-UNX did not show significant differences, so GH was dismissed as an important renotropic factor (Nicholson et al., 1979; Nomura et al., 1982).

A recent study that has taken the pulsatile nature of GH into account did find an early increase in pulsatile GH release after UNX in adult male rats (Haramati et al, 1994). In this study, the investigators found an increase in GH release 24 hours post-UNX. The increase was characterized by an increase in the amplitude of the GH surges and duration of release with peak GH levels 3-4 times greater compared with control animals. By 48 hours post-UNX, the GH concentration began declining (Haramati et al, 1994). Furthermore, when the increase in GH secretion was blocked by a GH release inhibiting factor, CRG was significantly attenuated (Haramati et al., 1994). These findings suggest that GH may be an important mediator of the initial stages of CRG following UNX in the adult animal.

The immature rat is in direct opposition to the adult rat as CRG is GH independent (Mulroney et al., 1992a). Therefore, age-related differences in the initiating stimuli for CRG are present and may also be species dependent.

It is known that GH is released in a pulsatile manner in the ovine fetus, and plasma concentration is several fold greater than in the adult sheep as both amplitude and baseline concentrations of GH are elevated in the fetus (Albers et al, 1993). In the ovine fetus, it is thought that SRIF does not inhibit further GH release in response to GRF until near term (de Zegher et al., 1989). The increased concentration of GH in the ovine fetus is thought to be due to unchecked activity of GRF, and maturation of the GH-GRF-SRIF axis is not complete until into the postnatal period (de Zegher et al., 1989)..

However, even though GH concentration is high in the fetus, GH is not thought to be an important mediator of fetal growth and development as anencephalic individuals are not growth retarded (Honnebier and Swaab, 1973). The apparent lack of effect of GH on in-utero growth was thought to be due to immaturity and/or lack of GH receptors in fetal tissues (Gluckman et al., 1983). A recent study using immunocytochemistry found GH receptors associated with the cell membranes of liver, pancreas, skin, cerebral cortex, and renal proximal and distal tubular epithelium in 14-16 week human fetuses (Hill et al., 1992). Studies in fetal rats have also demonstrated the presence of GH receptors and GH receptor mRNA, and specifically, GH receptor mRNA has been identified in the proximal straight tubule and the medullary thick ascending loop of Henle (Garcia-Aragon et al.; Chin et al, 1992).

It is still unknown if GH plays a direct role in fetal growth, but since GH receptors and GH receptor mRNA has been identified in fetal tissues, it remains a possibility that GH may have some role in fetal development. There are no studies in the literature addressing the role of GH in the acute response to UNX in the fetus. A study was undertaken to investigate the possibility that pulsatile secretion of GH was altered in response to UNX in the late gestational ovine fetus.

MATERIALS AND METHODS

Animal Instrumentation

All animals were subjected to health screening tests and acclimated as per Material and Methods in Part 2. Surgical instrumentation and manipulation were also performed as per Material and Methods in Part 2.

Sample Collection

Fetal blood (1½ mls) was collected from the femoral artery catheter every 20 minutes for 5 hours into heparin containing tubes. The withdrawn volume of blood was replaced with 3 times the amount of warm 0.9% NaCl. The blood tubes were placed on ice and spun in a refrigerated centrifuge at 4 C. Plasma from the tubes was removed and aliquoted into polypropylene tubes and frozen at -80 C for future GH analysis.

Radioimmunoassay of Growth Hormone

Plasma GH concentration was measured by double-antibody radioimmunoassay (RIA). All assays were performed in duplicate. Ovine GH (oGH) for iodination and primary (rabbit anti-sheep) antibody were courtesy of Dr. A.F. Parlow, National Hormone and Pituitary Program, National Institute of Diabetes and Digestive and Kidney Diseases.

Chloramine-T radioiodination of oGH was performed using 1 mCi of Na¹²⁵I (DuPont/NEN: NEZ-033A, Boston MA). All iodination reactions were carried out in the shipping vial for the Na¹²⁵I, and the iodination procedure performed under a fume hood approved for radionucleotide use. Ovine GH was reconstituted with 0.01 M NaHCO₃, pH 8.6 to achieve a final concentration of 0.1 µg/µl. Just prior to iodination, 100 µl of 0.5 M phosphate buffer (pH 7.5) was added to 20 µg of oGH and then the entire volume was added

to the Na^{125}I . Chloramine-T ($90\mu\text{g}$ in 0.05 M phosphate buffer) was added to the mixture and the tube gently rotated for 45 seconds. Sodium meta-bisulfite ($225\mu\text{g}$ in 0.05 M phosphate buffer) was added to the mixture and the tube gently rotated for 10 seconds. Following this, $100\mu\text{l}$ 10% BSA was added to the mixture. The entire mixture was placed on a Sephadex G-50 column ($20\text{ cm} \times 8\text{ cm}$ polypropylene, Kontes Scientific; Vineland, NJ) and eluted with 0.05 M barbital buffer pH 8.6 into borosilicate tubes containing $100\mu\text{l}$ 1% BSA-PBS. Approximately $500\mu\text{l}$ was eluted into each tube and a total of 36 tubes were collected.

Following elution, $2.5\mu\text{l}$ from each tube was aliquoted into separate borosilicate tubes and the gamma activity in the sample determined using a gamma counter (Cobra II/Auto-Gamma counting system, Packard Instrument Company; Downers Grove, IL). The results of the counts were graphed, and the first peak represented iodinated hormone. Iodinated hormone was aliquoted and stored at -20 C for future use.

Prior to use in the RIA, approximately 8×10^6 cpm of oGH was repurified over Sephadex G-100 through a $58\text{ cm} \times 0.8\text{ cm}$ borosilicate column. Ovine GH was eluted with barbital buffer and collected in 36 aliquots of 1 ml . The aliquots were counted on a gamma counter and the results plotted. Three peaks were obtained following elution, and the second peak represented repurified oGH which was used in the RIA.

Fetal plasma samples ($10\mu\text{l}$) were diluted to $200\mu\text{l}$ with 1% BSA-PBS, pH 7.5. Ovine GH for iodination was used to make assay standards so that the inhibition of binding of radiolabeled oGH to specific antibody by unknown samples could be compared with the inhibition by known standards. In borosilicate tubes, $200\mu\text{l}$ of standard or unknown was

added followed by 300 μ l of primary antibody (1:30,000), 500 μ l 1% BSA-PBS buffer, and 50 μ l of repurified oGH (20,000-40,000 cpm). The tubes were gently mixed and incubated at room temperature for 24 hours. Following incubation, 300 μ l of secondary antibody (sheep anti-rabbit) and 100 μ l 12.5% polyethylene glycol was added. The tubes were gently mixed and incubated for an additional 2 days at room temperature. The tubes were then spun at 3500 rpm for 30 minutes at 4 C. The supernatant was discarded, and the radioactivity of the precipitate containing bound Na¹²⁵I determined in a gamma counter.

Nonspecific binding was < 5% in all assays and total binding averaged 40% for all assays. Coefficient of variation within and between assays calculated on replicates of fetal plasma was 8.78 and 12.5%, respectively.

Statistical Analysis

Analysis of pulsatility of hormone secretion was performed employing the computer algorithm Cluster (provided by Dr. J Veldhuis, University of Virginia, Charlottesville, VA). Cluster analysis was also used to measure the mean plasma concentration of oGH and the total amount of oGH secreted over the 5 hour study period (Veldhuis and Johnson, 1986). Total hormone secreted was calculated based on total area under the curve for the duration of the experiment.

Significant differences between the means of the control (sham operated) and nephrectomy groups were determined by Student's *t* test. Significance was determined at the $P \leq 0.05$ level. SigmaStat TM statistical software was used for all statistical analysis (Jandel Scientific, San Rafael, CA).

RESULTS

Plasma Growth Hormone Concentration

24 hours post-uninephrectomy

Table 1¹ shows the change in plasma GH concentration 24 hours post-UNX. Values are expressed as mean $\pm$ standard error of the mean (SEM). Growth hormone concentration in the control group was 135.7 ± 14.7 ng/ml, and concentration in the nephrectomy group was 127.7 ± 13.6 ng/ml. The total GH secreted over the 5-hour study period in the control group was 40893.2 ± 4491.6 ng/5 hrs, and the total secreted in the nephrectomy group was 38163.4 ± 4013.3 ng/5 hrs. Differences between the groups were not significant. The pattern of hormone pulsatility between the 2 groups did not change and the mean pulse interval for both groups was approximately 2-2½ hours.

72 hours post-uninephrectomy

Table 2 shows the change in plasma GH concentration 72 hours post-UNX. Values are expressed as mean $\pm$ SEM. Growth hormone concentration in the control group was 156.3 ± 17.9 ng/ml, and concentration in the nephrectomy group was 128.4 ± 17.8 ng/ml. The total GH secreted over the 5-hour study period in the control group was 38463.8 ± 5387.1 ng/ml, and the total secreted in the nephrectomy group was 46815.2 ± 5321.0 ng/ml. Differences between the groups were not significant. The pattern of hormone pulsatility between the 2 groups did not change and the mean pulse interval for both groups was approximately 2-2½ hours.

¹ All Tables appear after the Discussion section.

DISCUSSION

The results of this study show that UNX in the late term ovine fetus has no effect on pulsatile growth hormone secretion. These results are similar to the findings of Mulroney et al. in which no increase in GH secretion was found 24 hours post-UNX in immature male rats (Mulroney et al., 1992a). Additionally, these investigators administered an antagonist to GH-releasing factor and found CRG of the remnant kidney still occurred despite a reduction in whole body gain (Mulroney et al., 1992a). This is in contrast to the findings in adult male rats in which administration of a GH-releasing factor antagonist significantly reduced CRG post-UNX (Haramati et al, 1994). Therefore, the conclusion drawn from these studies is that it appears as if GH is not an important mediator in the acute phase of CRG in the fetal or immature animal.

The role of GH in CRG in the immature or fetal animal cannot be ruled out as GH may be working through local renal tissue effects without an increase in circulating levels. This scenario would be feasible if renal tissue sensitivity to GH were increased post-UNX, leading to increased local production of insulin-like growth factor I (IGF-I). It is known that the circulating or endocrine form of IGF-I is translated from exon 2 of IGF-I mRNA, and is released primarily from the liver in response to GH (Chatelain et al., 1991; LeRoith et al., 1993). This relationship is the basis for the GH-IGF-I axis. However, the situation with tissue (autocrine or paracrine) IGF-I is less clear. Evidence for autocrine or paracrine IGF-I includes the presence of mRNA for IGF-I and production of IGF-I in many tissues. Also, levels of IGF-I and IGF-I mRNA vary in different tissues, and control of IGF-I synthesis by GH occurs only in the liver (Chatelain et al., 1991).

In the weanling rat, it has been shown that renal tissue IGF-I production is GH independent, a finding that has also been shown in several tissues, including the kidney, in young (6 months of age) sheep (Mulroney et al., 1992b; Hua et al., 1993). These findings are different from traditional dogma that held that tissue IGF-I was strongly GH dependent. It has been demonstrated in the fetus that IGF-I and IGF-II are not GH dependent because in anencephalic fetuses, concentrations of IGF-I and IGF-II are not different from normal individuals (Hill et al., 1992).

The possibility that IGF-I may be responsible for early CRG in the immature animal has been demonstrated by Mulroney et al. These investigators were able to demonstrate significant increases in renal IGF-I mRNA, IGF-I peptide, IGF-I receptor, and IGF-I ligand binding 24 and 48 hours post-UNX in the weanling male rat (Mulroney et al., 1991; Mulroney et al., 1992a; Mulroney et al., 1992b). The increase in renal IGF-I mRNA preceded induction of the early response genes *c-fos* and *c-jun* and subsequent mitogenic response by several hours, so IGF-I may be an integral part of induction of early response gene mediated hyperplastic CRG in the immature animal (Mulroney et al., 1996). Again, the response in the immature animal is different from the adult as neither renal IGF-I mRNA nor early response genes were increased during early hypertrophic CRG in response to UNX in the adult rat (Mulroney et al., 1996). However, until a specific in-vivo antagonist to IGF-I is available, it will remain purely speculative that IGF-I is an initiator of CRG in the immature animal.

Experiments concerning CRG and IGF-I in the fetus have not been performed. In light of the findings in the rat, it might be expected that the fetus would be similar to the

weanling rat. However, until such experiments are performed, this remains open to speculation. Preliminary results of such studies in our laboratory indicate no increase in expression of IGF-I mRNA in the remnant kidney in the late gestational ovine fetus in response to UNX (unpublished observations).

Both IGF-I and IGF-II have been demonstrated to be important in the development of the metanephric rat kidney (Rogers et al., 1991). Both IGF-I and IGF-II were synthesized and released by the metanephric rat kidney and mRNA for both were also isolated. Concentration of IGF-II released into the culture media of the metanephric kidney was two times greater than IGF-I. However, monoclonal antibodies to either IGF-I or IGF-II prevented growth and differentiation of metanephroi in vitro (Rogers et al., 1991). This study suggests that both IGF-I and IGF-II are produced within the developing metanephric kidney and both are necessary for renal growth and development to occur. So, it may be that CRG in the fetus is dependant upon increases in IGF-I and IGF-II. To date, no studies have been performed in the fetus investigating the role of IGF-I and IGF-II in CRG.

It appears that the initiation of CRG in the late term ovine fetus is GH independent. However, administration of a GH release inhibiting factor along with UNX would be necessary to fully discount GH as an initiator of CRG in the late term ovine fetus.

Table 1. Change in Plasma Growth Hormone Concentration 24 Hours Post-Uninephrectomy.

Group	n	Mean Growth Hormone Concentration (ng/ml)	Total Growth Hormone Secreted (ng/5 hrs)
Control	6	135.7 ± 14.7	40893.2 ± 4491.6
Nephrectomy	7	127.7 ± 13.6	38163.4 ± 4013.3

Table 2. Change in Plasma Growth Hormone Concentration 72 Hours Post-Uninephrectomy.

Group	n	Mean Growth Hormone Concentration (ng/ml)	Total Growth Hormone Secreted (ng/5 hrs)
Control	7	156.3 ± 17.9	38463.8 ± 5387.1
Nephrectomy	7	128.4 ± 17.8	46815.2 ± 5321.0

LITERATURE CITED

- Addis T. The ratio between the urea content of the urine and of the blood after the administration of large quantities of urea: An approximate index of the quantity of actively functioning kidney tissue. *J Urol.* 1:263-287, 1917.
- Albers N, Bettendorf M, Herrmann H et al. Hormone ontogeny in the ovine fetus. XXVII. Pulsatile and copulsatile secretion of luteinizing hormone, follicle-stimulating hormone, growth hormone, and prolactin in late gestation: A new method for the analysis of copulsatility. *Endocrinology.* 132:701-709, 1993.
- Astarabadi TM, Essex HE. Effect of hypophysectomy on compensatory renal hypertrophy after unilateral nephrectomy. *Am J Physiol.* 173:526-534, 1953.
- Astarabadi T. The effect of growth and lactogenic hormone on renal compensatory hypertrophy in hypophysectomized rats. *Q J Exp Physiol.* 48:85-92, 1963a.
- Astatabadi T. The effect of hypophysectomy, adrenalectomy and ACTH administration on compensatory renal hypertrophy in rats. *Q J Exp Physiol.* 48:80-84, 1963b.
- Buonomo FC, Baile CA. The neurophysiological regulation of growth hormone secretion. *Domest Anim Endocrinol.* 7:435-450, 1990.
- Chatelain P, Naville D, Avallet O et al. Paracrine and autocrine regulation of insulin-like growth factor I. *Acta Paediatr Scand Suppl.* 372:92-95, 1991.
- Chin E, Zhou J, Bondy CA. Renal growth hormone receptor gene expression: Relationship to renal insulin-like growth factor system. *Endocrinology.* 131:3061-3066, 1992.
- de Zegher F, Daaboul J, Grumbach M et al. Hormone ontogeny in the ovine fetus and neonate. XXII. The effect of somatostatin on the growth hormone (GH) response to GH-releasing factor. *Endocrinology.* 124:1114-1117, 1989.
- Dicker SE, Greenbaum AL, Morris CA. Compensatory renal hypertrophy in hypophysectomized rats. *J Physiol (Lond).* 273:241-254, 1977.
- Fine LG. The biology of renal hypertrophy. *Kidney Int.* 29:619-634, 1986.
- Johnson HA, Vera Roman JM. Renal epithelial hyperplasia: failure to demonstrate a humoral control factor. *Cell Tiss Kinet.* 1:35-41, 1968.
- Gluckman PD, Butler JH, Elliott TB. The ontogeny of somatotrophic binding sites in ovine hepatic membranes. *Endocrinology.* 112:1607-1612, 1983.

Haramati A, Lumpkin MD, Mulroney SE. Early increase in pulsatile growth hormone release after unilateral nephrectomy in adult rats. *Am J Physiol.* 266:F628-F632, 1994.

Hill DJ, Riley SC, Bassett NS, et al. Localization of the growth hormone receptor, identified by immunocytochemistry, in second trimester human fetal tissues and in placenta throughout gestation. *J Clin Endocrinol Metab.* 75:646-650, 1992.

Honnebier WJ, Swaab DF. The influence of anencephaly upon intrauterine growth of the fetus and placenta and upon gestation length. *J Obstet Gynaecol Br Commonw.* 80:577-588, 1973.

Hua KM, Ord R, Kirk S et al. Regulation of plasma and tissue levels of insulin-like growth factor-I by nutrition and treatment with growth hormone in sheep. *J Endocrinol.* 136:217-224, 1993.

Katz AI, Toback FG, Lindheimer MD. The role of renal "work" in compensatory kidney growth. *Yale J Biol Med.* 51:331-337, 1978.

LeRoith D, Kavsan VM, Koval AP et al. Phylogeny of the insulin-like growth factors (IGFs) and receptors: A molecular approach. *Mol Reprod Dev.* 35:332-338, 1993.

Lowenstein LM, Stern A. Serum factor in renal compensatory hyperplasia. *Science.* 142:1479-1480, 1963.

MacKay LL, MacKay E, Addis T. Factors which determine renal weight. *J Nutr.* 4:379-383, 1931.

Meyer TW, Baboolol K, Brenner BM. Nephron adaptation to renal injury. In: *The Kidney.* Brenner BM ed. WB Saunders Company, Philadelphia. 2:2011-2048, 1996.

Mulroney SE, Haramati A, Roberts CT Jr et al. Renal IGF-1 mRNA levels are enhanced following unilateral nephrectomy in immature but not adult rats. *Endocrinology.* 128:2660-2662, 1991.

Mulroney SE, Lumpkin MD, Roberts CT Jr et al. Effect of a growth hormone-releasing factor antagonist on compensatory renal growth, insulin-like growth factor -I (IGF-I), and IGF-I receptor gene expression after unilateral nephrectomy in immature rats. *Endocrinology.* 130:2697-2702, 1992a.

Mulroney SE, Haramati A, Werner H et al. Altered expression of insulin-like growth factor-I (IGF-I) and IGF receptor genes after uninephrectomy in immature rats. *Endocrinology.* 130:249-256, 1992b.

Mulroney SE, Koenig JI, Csikos T et al. Temporal changes in insulin-like growth factor I, *c-fos* and *c-jun* gene expression during hyperplastic kidney growth in weanling rats. *Endocrinology*. 137:839-845, 1996.

Nicholason WE, Barton RN, Puett D et al. Evidence for renotropic activity in ovine pituitaries. *Endocrinology*. 105:16-20, 1979.

Nomura K, Puett D, Nicholason WE et al. Partial purification and characterization of a renotropic fraction from ovine pituitaries. *Proc Natl Acad Sci USA*. 79:6675-6679, 1982.

Ogawa K, Nowinski WW. Mitosis stimulating factor in serum of unilaterally nephrectomized rats. *Proc Soc Exp Biol Med*. 99:350-354, 1958.

Preuss HG, Goldin H. A renotropic system in rats. *J Clin Invest*. 57:94-101, 1976.

Reiter RJ, McCreight CE. Failure to demonstrate a humoral factor controlling compensatory renal hyperplasia. *Anat Rec*. 148:396-397, 1964.

Rogers SA, Ryan G, Hammerman MR. Insulin-like growth factors I and II are produced in the metanephros and are ecarte for growth and development in vitro. *J Cell Biol*. 113:1447-1453, 1991.

Royce PC. Inhibition of renal growth following unilateral nephrectomy in the rat. *Proc Soc Exp Biol Med*. 113:1046-1049, 1963.

Sacerdotti C. Ueber die compensatorische hypertrophie der nieren. *Virchows Arch Pathol Anat*. 146:267-297, 1896.

Strobl JS, Thomas MJ. Human growth hormone. *Pharm Rev*. 46:1-34, 1994.

Veldhuis JD, Johnson ML. Cluster analysis: A simple, versatile and robust algorithm for endocrine pulse detection. *Am J Physiol*. 250:E486-E493, 1986.

Weinman EJ, Renquist K, Stroup R et al. Increased tubular reabsorption of sodium in compensatory renal growth. *Am J Physiol*. 224:565-571, 1973.

PART 5
SUMMARY

SUMMARY OF PARTS 2-4

This study accomplished all the research aims set forth in Part 1. By performing left unilateral nephrectomy (UNX) on late gestational (> 130 days gestation, nephrogenesis complete) fetal sheep and using the chronic sheep preparation, we were able to study the in-utero response of the animal. Standard clearance techniques were used to document glomerular filtration rate (GFR) in UNX animals and in age-matched sham operated (control) animals. Additionally, functional parameters of fractional clearance of urinary electrolytes and urine flow rate were measured.

The results indicated that the fetal sheep did not undergo an absolute increase in GFR 24 or 72 hours post-UNX as evidenced by a significant decrease in GFR in mls/min or mls/min/kg body weight compared with control animals. However, there was no difference in GFR between the groups when comparing GFR in mls/min/gm total kidney weight, and GFR in mls/min/gm right kidney weight was significantly greater in UNX animals 24 and 72 hours post-UNX. This demonstrated that the remaining functional renal tissue was performing at a higher level compared with control animals.

It is expected that fractional clearance of electrolytes will increase following an acute reduction in renal mass. The results of this study confirmed that this occurs in the fetus 24 hours post-UNX as fractional clearance of sodium, potassium, chloride, and phosphate were all increased, although not significantly. By 72 hours post-UNX, fractional clearance of electrolytes had decreased and values were very similar to control animals. This restoration of glomerulotubular balance occurred very quickly as reports in postnatal animals indicate that it takes 2-4 weeks for this response to be complete.

We were unable to document an increase in urine flow rate 24 or 72 hours post-UNX. An increase in urine flow rate accompanied by an increased electrolyte loss in the urine is the usual response to an acute reduction in renal mass in postnatal animals. Hydration status of the ewe and/or fetus, or changes in systemic blood pressure or intrarenal blood flow may account for this finding.

It appears as if the late gestational ovine fetus responds very differently from the postnatal animal in response to an acute reduction in renal mass. Absolute GFR does not increase as is the case in the postnatal animal, but GFR per amount of functioning renal tissue does increase. Also, glomerulotubular balance is restored much faster, and urine flow rate does not increase.

Assessment of the magnitude and type of compensatory renal growth (CRG) following UNX in the fetus demonstrated striking differences in comparison to postnatal animals. The data did not indicate an increase in kidney weight of the remnant kidney compared with age-matched controls 24 or 72 hours post-UNX. Although by 72 hours post-UNX the renoprival had increased in total weight 8.5% above the normal growth demonstrating a trend toward CRG. Also, the kidney weight to body weight ratio in the remnant kidney was significantly greater than the ratio in the extant kidney 72 hours post-UNX. This finding is in contrast to both immature and adult animals in which UNX results in significant CRG within 24 hours. Other studies in fetal animals have found conflicting results to UNX. It is possible that the response to UNX in the fetus depends on when UNX was performed in relation to the completion of nephrogenesis. Other studies in fetal sheep demonstrating CRG in response to a reduction in renal mass were performed prior to

completion of nephrogenesis. It may be that the fetal kidney responds maximally to a reduction in renal mass when the perturbation occurs prior to completion of nephrogenesis. Also, had the current study examined the response to UNX over a protracted time course, CRG may have been demonstrable.

By 72 hours post-UNX, an increase in remnant kidney DNA and protein content over the extant kidney was demonstrated. As a percent increase, the increase in protein (51%) was greater than the increase in DNA (40%) demonstrating that hypertrophy predominated over hyperplasia. This is in sharp contrast to immature animals in which hyperplastic CRG primarily occurs, and is more similar to adult animals in which hypertrophic CRG primarily occurs. Had the animals been followed for a longer time post-UNX, and increase in DNA and/or protein content of the remnant kidney over control values may have been documented.

Growth hormone (GH) was examined as a potential initiator of the adaptive response to UNX. In rats, it is known that pulsatile GH secretion increases in the acute phase following UNX in adult but not immature rats. The role of GH as a potential mediator of the compensatory adaptation in the acute phase following UNX in the fetus was documented in this study. No difference in mean GH secretion or in total GH secretion was found 24 or 72 hours post-UNX versus controls in the late gestational ovine fetus. The pulsatile nature of GH secretion was also not different between the two groups. This finding is not surprising in view of the lack of effect that GH has on the developing fetus. It is still possible that the GH-insulin-like growth factor- I (IGF-I) axis may play a role in the compensatory response to UNX. Preliminary results from experiments in this area have not shown an increase in IGF-I mRNA in remnant kidneys 24 or 72 hours post-UNX in the ovine fetus.

LIMITS OF THE PARADIGM

The advent of the chronic sheep preparation has allowed the science of fetal renal physiology to greatly advance. However, there are some important limits to the model that must be considered. First, in any study examining change in fetal growth, normal growth must first be established in order to make meaningful statements about an increase in growth. This is difficult to do in the fetus because of the great variability in individuals. The presence of twins and even triplets complicates the matter even more in the sheep. In this study, only single pregnancies were used for UNX animals so that the presence of other fetuses would have no compensatory effect on the UNX fetus. Control animals were single, twin, or triplet pregnancies so even though the controls were age-matched to the UNX group, the comparison between the groups was not an exact 1 for 1 comparison.

An additional consideration of using this model is stress. Even though chronic catheterization allows the study of the animal away from the acute stress of surgery and anesthesia, studies done within the first few days following the catheterization procedure are still subject to post-operative stress on the animal. The sheep as a research animal is somewhat problematic in this regard as well because by nature, they are easily stressed. We tried to minimize this aspect in our studies by performing them in a quiet room isolated from other distractions, and by providing the ewe free accesses to food throughout the study period. However, temperament of the animals was extremely variable, and some ewes were obviously stressed during the study period.

A final limit of the chronic preparation is the failure rate. In this study, failure rate due to catheter failure, lack of urine production by the fetus, non-physiologic fetuses, and

lambing prior to study was approximately 25%. This rate is not extremely high for the chronic sheep preparation, but is higher than studies in postnatal animals. Much detail must be paid to proper handling and maintenance of the catheters in order to keep them patent and prevent ascending infection to the fetus. Careful attention must also be given to the ewe in the post-operative period, and any complications treated quickly to prevent loss of the fetus and/or ewe. Monitoring of the ewe and fetus are easily accomplished by blood gas analysis of both and by physical examination of the ewe, but both are highly labor intensive.

The chronic sheep preparation is a useful method to study perturbation of the fetus in-utero, but the limits of the model must be kept in mind when interpreting results of such studies. By using meticulous surgical technique and careful attention to blood gas status of the fetus during surgery, fetal stress can be minimized. This along with physiologic monitoring of the fetus and ewe in the post-operative period ensures that only fetuses in the physiologic range are used for study. Any fetus that is not physiologic should be excluded from study.

VITA

Hilary K. Matthews was born in Zanesville, Ohio on May 7, 1962. She was graduated from Brecksville-Broadview Heights Senior High School in May 1980. She was graduated cum laude from Wittenberg University in Springfield, Ohio in June 1984 with a Bachelor of Arts degree in Biology and Psychology. She then attended The Ohio State University College of Veterinary Medicine, Columbus, Ohio and was graduated cum laude with a Doctor of Veterinary Medicine degree in June 1988. In July of 1988, she began a one-year internship in large animal medicine and surgery at North Carolina State University in Raleigh, North Carolina. Following completion of her internship, she began a three-year residency in large animal internal medicine at The University of Tennessee at Knoxville. After completion of her residency in 1992, she became board certified by The American College of Veterinary Internal Medicine. In 1992 she began to work as a postdoctoral research associate in the Department of Pediatrics at The University of Tennessee Medical Center, Knoxville, Tennessee, and was admitted to the Comparative and Experimental Medicine Program to pursue her doctoral degree. The doctoral degree was received December 1996.